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PRINCIPAL INVESTIGATOR: Philip G. Koehler
Richard S. Patterson

CONTRACTING ORGANIZATION: University of Florida
Gainesville, FL 32611-0110

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Philip G. Koehler; Richard S. Patterson

E-Mail: pgk@ufl.edu

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14. ABSTRACT
Filth flies serve as vectors for many diseases that pose a serious threat to the safety and well-being of deployed military personnel. Our research project targeted the development of new insecticides for fly control. During the 3-year research project, research on control of mosquitoes and flies developed from the initial screening of insecticidal active ingredients to a filed testing of new formulation and new application devices. We obtained several insecticides including pyrethroids, neonicotinoids, phenylpyrazoles, oxadiazines, and organophosphates and screened them against flies. We tested fly traps and light traps to optimize military usage of these non-chemical controls. A sprayable spot fly bait was evaluated and proved to be very useful for use by deployed troops. After our studies and recommendation the product received NSN (01-555-9369) and is available for use by military entomologists. New volatile compounds were tested against both flies and mosquitoes, and demonstrated to be useful for control of mosquitoes and flies in confined areas. Insecticide-impregnated wool cords were shown to be the best material for delivery of the insecticides to flies, because an efficient acquisition of pesticide by the insects from wool cords, possibly due to presence of natural oils. The grant supported six graduate students, three of them military entomologists.

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1. INTRODUCTION

Flies serve as vectors for many diseases that pose a serious threat to the safety and well being of deployed military personnel. They transmit many enteric diseases including dysentery, cholera, and typhoid fever. Biting flies like sand flies, biting midges, stable flies, horse and deer flies are important vectors of diseases such as anthrax, tularemia, and leishmaniasis. In addition to spreading disease, filth flies like house flies, blow flies, flesh flies, and other Muscoid flies can affect the readiness of troops for military action by reducing efficiency of personnel and affecting morale. In sum, they are a major problem for armed forces deployed for combat.

Combat troops often have to contend with a number of different species of flies. Filth flies are a major problem anytime there is a military action, because commonly there is an absence or disruption of sanitary systems and governmental services. The rapid deployment of troops places stress on the military supply distribution system. Frequent unit movements and other factors make it difficult for fly control supplies to be delivered to the units.

Treatments to control filth flies, that are usually active during daytime, often require military personnel to apply baits or sprays to knock down heavy populations. Biting flies are usually even more mobile than mosquitoes, and present a different problem for troop protection in deployed areas. In both cases, deployed troops need treatments for fly control that minimize or eliminate exposure to insecticides.

Flies also pose a severe risk in deployed hospital environments. House flies, flesh flies and other Muscoid Diptera are known to be attracted to sweat, saliva, blood, and serum. They can land on wounds, lay their eggs, and cause myiasis. As a result, they can easily contaminate field deployed medical facilities and injured troops.

Flies are very difficult to control in situations where troops are being deployed or in mobile combat arenas, and insecticides used to control infestations may harm troops. However, chemical control is still the most important element in an integrated approach to vector control. Three general methods have been used to reduce problems caused by Muscoid flies: preventing breeding, excluding adult flies with screens or other barriers, or killing flies before they can cause harm or reproduce.

Our research project targets the concept of killing adult flies before they can cause harm or reproduce. By merging the developments in insecticides with new modes of action with traditional concepts of delivery using our knowledge of fly behavior, we expect to modify and develop, in conjunction with industry, new technologies of fly control that will successfully solve fly problems for deployed military. For instance, flies prefer to rest on strings and cords and can be controlled using insecticide treated yarn (Hogsette and Ruff 1996). They also are attracted to surfaces that reflect ultraviolet light (Patterson et al. 1980, Patterson and Koehler 1982. Patterson et al. 1981). It should be possible to treat insecticide-impregnated cords or yarn with ultraviolet light reflective dyes. These cords or yarns could be employed near troops to kill flies quickly. Also, baits do not need to be formulated as scatter baits; they can be formulated as bait-treated surfaces. These baited surfaces could be activated by peeling off protective coverings and be hung in areas where flies occur. By using new classes of insecticides, insecticide resistance in fly populations is expected to be overcome. New fly control technologies will provide better protection than products and technologies currently used by the military to suppress flies when deploying troops. Because the number of different insecticides and

mechanisms for their use in military conditions remains very limited, more options are needed for effective vector control programs.

2. RESEACH ACCOMPLISHMENTS FOR YEAR 3:

C. Students

LTJG Jeff Hertz (USN), M.S. Jeff evaluated toxicants applied to cords for fly control. He discovered that flies preferred to rest on manila cord; however, best kill with fipronil and indoxacarb was on wool cord. His results were presented at the AFPMB as part of the justification for request of National Stocking Number (NSN) for a new sprayable and paintable fly bait product. The request was approved and the product received NSN 01-555-9369.

HM1 Joseph Diclaro (USN), M.S. Candidate. Joe joined the laboratory in April 2007. He is currently working with control of house flies using cord and trap combinations, which are a continuation of the work previously developed by LTJG Hertz. His work involves the development of novel application devises for control of filth flies. Joe represented LTJG Hertz and the Univ. of Florida – Urban Entomology research team at the AFPMB when the NSN request for the new sprayable spot fly bait was presented to the pesticide committee.

Lt. Ricky Vazquez (U. S. Army Reserves), Ph.D. candidate, Ricky recently returned from deployment in Iraq. His work was interrupted when he was activated and transferred to Iraq so some of the work planned for him was completed by Jeff Hertz. Currently, Ricky is working with biology and control of invasive ant species.

Alexandra Chaskopoulou, M.S. Alex evaluated vapor toxicity of low molecular weight chemicals on flies and mosquitoes. She adapted a bioassay for testing vapor toxicity to flies and mosquitoes and has completed her evaluation of 3 volatile compounds against mosquitoes, including evaluation of a controlled release system for application of volatile compounds. These novel compounds can replace the organophosphate dichlorvos without the harmful effects displayed by dichlorvos.

Ryan Welch, M.S. Ryan evaluated of fly attractants in commercially available cone traps and determined optimal configurations of trap design and attractant age. He submitted his thesis and graduated in December 2006. Currently, he is working for McCall's Services in Jacksonville, FL as a manager trainee.

Matt Aubuchon, Ph.D. Worked on attraction of flies to light. Light traps are the major technique used in military mess facilities to reduce numbers of flies in food handling, serving, and eating areas. His work developed procedures for using light traps more effectively. Matt submitted his dissertation and graduated in May 2006. He is currently coordinator of the Deployed War-Fighter Protection Project at the Center for Medical, Agricultural and Veterinary Entomology (CMAVE) USDA-ARS in Gainesville, FL.

D. Most Recent Research Results

I. Wind Tunnel for Testing Insecticides

The research planned for the wind tunnel was redirected to testing of volatile compounds in laboratory setting, and the wind tunnel was not used further. Delays in construction and mounting the wind tunnel dictated redirection but the tunnel continues to be available for future testing of compounds against flies and mosquitoes. Initial discussions with USDA and NECE researchers raised the possibility of using the wind tunnel with new compounds being tested at CMAVE and NECE.

II. Vapor Toxicity of Volatile Compounds to Mosquitoes

Materials and Methods

Chemicals and Insects. Fifteen novel insecticides were tested; 7 formate esters [ethylene glycol di-formate (EGDF), methyl formate, ethyl formate, propyl formate, butyl formate, hexyl formate and heptyl formate) (Table 1), 4 heterobicyclic esters (menthofuran, benzothiophene, coumaran and dimethyl-coumarone) (Table 2), and 4 acetate esters (propyl acetate, butyl acetate, pentyl acetate and hexyl acetate) (Table 3). Dichlorvos (DDVP) was tested as a positive control. Mosquitoes used were the USDA-CMAVE Orlando strain of *Aedes aegypti* (L.). Ten females were used per bioassay jar and a minimum of 300 mosquitoes was exposed to each insecticide.

Bioassay Main bioassay. Ten females were transferred into 125 ml vials covered with window screening (~1.55 mm mesh) to prevent insect escape while allowing for gas exchange. Insects were provided a cotton wick dipped in 10% sugar solution. Vials with mosquitoes were placed individually in 1-liter Mason jars along with a filter paper disk (55 mm in diameter) (Fig. 1). Insecticide solutions were applied to the filter paper and the jars were closed rapidly and tightly to prevent vapor escape. After a 24 h exposure, mortality was recorded.

DEF and PBO bioassay. This bioassay was similar to the one above with an extra step to expose mosquitoes DEF or PBO, which were applied to the mosquito vials before the transfer of the insects into them.

Data Analysis. Where appropriate mortality adjusted using the Abbott's formula and LC_{50} and LC_{90} were estimated using probit analysis and compared using 95% confidence limits. Average insect weight was used to adjust dose from mg/liter of air to mg/g of insect body weight/liter of air.

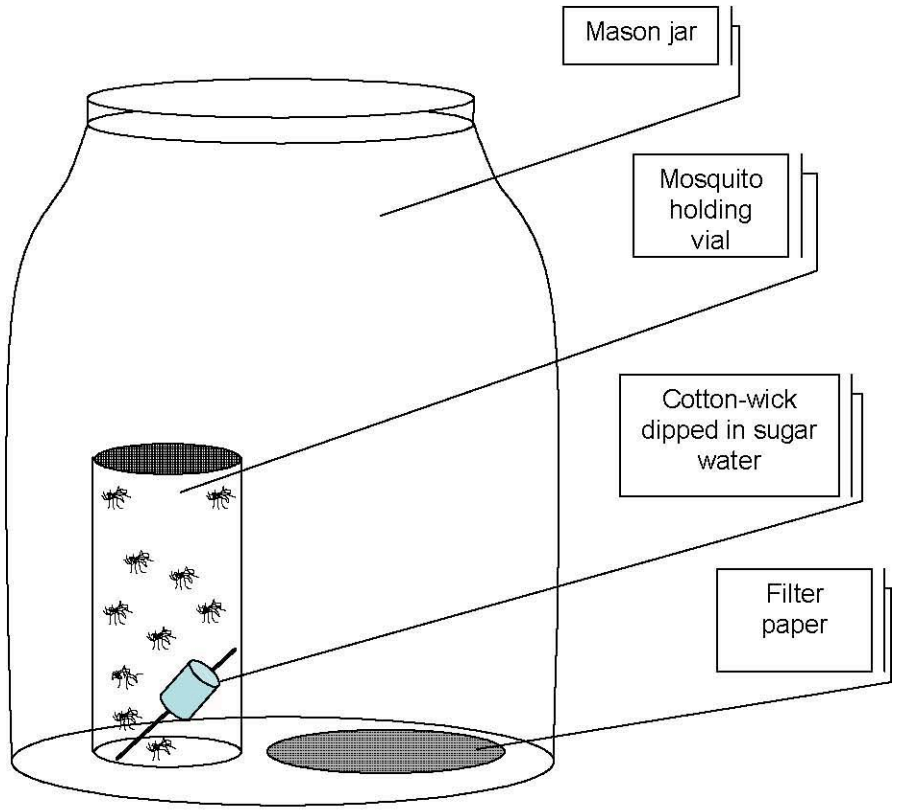

Figure 1. Main mosquito and fly bioassay set-up.

Results

Toxicity of Novel Compounds. DDVP was by far the most toxic compound followed by methyl formate (Table 4, Fig. 2). Formate esters were the most toxic family followed by the heterobicyclics and the acetate esters. Methyl formate was the most toxic ester (LC_{50} = 1.36 mg/liter) and was heptyl formate (LC_{50} = 3.17 mg/liter). Coumaran was the most toxic heterobicyclic (LC_{50} = 2.03 mg/liter), and menthofuran, was the worst heterobicyclic. The acetate esters hexyl acetate was the least toxic compound (LC_{50} = 5.09 mg/liter). DEF decreased toxicities of formate esters by 1.4 to 2.6 X, and PBO increased menthofuran toxicity (Table 5, Fig. 3).

Role of Volatility in Toxicity. Molecular weight, density, and boiling point were investigated as predictors of volatility. For the formate esters the regressions of LC_{50} versus molecular weight, boiling point, and density were correlated with R^2 = 0.57, 0.69, and 0.19, respectively. For the heterobicyclics the regression of LC_{50} versus molecular weight was correlated with R^2 = 0.88, and the regressions of LC_{50} versus density and boiling point had R^2 < 0.25. For the acetate esters the regressions of LC_{50} versus molecular weight, density and boiling point were weak (R^2 = 0.24, 0.20, and 0.24 respectively). When combined families regression was performed there was a poor correlation between toxicity and all 3 volatility predictors, except maybe with molecular weight (R^2 = 0.5).

Toxicities of Novel Compounds to Mosquitoes and *Drosophila* There were significant

differences among the toxicities of the compounds on mosquitoes and *Drosophila* when body-weight corrected LC_{50} values (in mg /liter/g of insect per liter) were compared (Table 6). DDVP was by far the most toxic insecticide for both insects and was significantly more toxic to mosquitoes than *Drosophila*. All compounds, except for menthofuran, were significantly more toxic to mosquitoes than *Drosophila*. On average the 14 compounds were approximately 3.5 times more toxic to mosquitoes, whereas menthofuran was 1.7 times more toxic to *Drosophila*. On mosquitoes, formates showed highest toxicities, followed by heterobicyclics and acetates. On *Drosophila*, acetate esters consistently showed poor toxicity. The best 7 performing compounds on mosquitoes were methyl, butyl, propyl, ethyl, and hexyl formate, and the heterobicyclics coumaran, and benzothiophene. The most toxic compound on *Drosophila,* menthofuran, was one of the least toxic compounds against mosquitoes. The most toxic compound on mosquitoes, methyl formate, was one of two least toxic compounds against *Drosophila*.

Table 1. Physical and chemical properties of formate esters.

Formate Esters	Mol. Weight	bp (°C)	Density (g/ml)	Natural occurrence	Used as	Other Properties
Methyl formate	60.05	33	0.974	–	-Quick drying finishes - Alternative to sulfur dioxide in domestic refrigerators	Clear liquid with an ethereal odor
Ethyl formate	74.08	53	0.921	–	Flavoring agent (raspberries flavor)	Characteristic smell of rum
Propyl formate	88.11	80.5	0.904	Apple, Pineapple, Plum, Currant	Flavoring agent (brandy & rum products)	Colorless liquid with a sweet fruity/berry odor
Butyl formate	102.13	106.5	0.892	Pear	Flavoring/odor agent (rum, pear, plum products)	Colorless liquid with a fruity/green odor
Hexyl formate	130.18	155.5	0.879	Pear	Flavoring/odor agent (apple, banana, lemon, strawberry, orange products)	Colorless liquid with a medium fruity odor
Heptyl formate	144.21	178	0.882	Kumquat	Flavoring/odor agent (apple, apricot, coconut, kumquat, peach, rose, wine products)	Colorless liquid with a medium green/floral/ apple scent
Ethylene glycol diformate	118.09	176	1.226	–	–	Colorless, odorless liquid

Table 2. Physical and chemical properties of heterobicyclics.

Heterobicyclic Esters	Mol. Weight	bp (°C)	Density (g/ml)	Natural occurrence	Used as	Other Properties
Menthofuran	150.22	205	0.97	Peppermint oil	Flavoring/odor agent (chocolate, coffee, peppermint)	Bluish clear liquid with a musty nutty/coffee odor
benzothiophene	134.20	221.5	1.149	Constituent of petroleum related deposits (lignite tar)	Found in the chemical structure of pharmaceutical drugs for treating osteoporosis & asthma (raloxifen, zileuton)	Solid crystalline form with an odor similar to naphthalene
Coumaran	120.15	188.5	1.065	_	Found in the chemical structure of pharmaceutical drugs (insomnia treatments)	_
Dimethyl-coumarone	146.19	101.5	1.034	Cade oil Tobacco Coffee	Flavoring/odor agent (chocolate, coffee, tobacco, vanilla, leather products)	Pale yellow liquid with a strong phenolic odor

Table 3. Physical and chemical properties of acetate esters.

Acetate Esters	Mol. Weight	bp (°C)	Density (g/ml)	Natural occurrence	Used as	Other Properties
Propyl acetate	102.3	102	0.888	–	Flavoring/odor agent	Clear colorless liquid with an odor of pears
Butyl acetate	116.16	125	0.88	Several fruits (e.g., Apples in the Red Delicious variety)	Flavoring agent (candy, ice-cream, cheeses, baked goods)	Colorless liquid with a fruity odor
Pentyl acetate	130.18	146	0.876	–	–	Colorless liquid with an odor similar to banana odor
Hexyl acetate	144.21	169	0.87	–	Flavoring and fragrance agent	Colorless liquid with a fruity/pear odor

Table 4. Vapor toxicities of 15 novel, low molecular weight, volatile compounds and the organophosphate DDVP to mosquitoes *Aedes aegypti* (L.).

Insecticide Families Insecticides	Slope ± SE	LC_{50} mg/liter (95% CI)	LC_{90} mg/liter (95% CI)	$\chi 2$	P
Organophosphates					
DDVP[a]	4.84 ± 0.51	0.025 (0.023-0.027)	0.047 (0.042-0.056)	4.50	0.11
Formate esters					
Methyl formate	9.84 ± 1.10	1.36 (1.311-1.40)	1.83 (1.74-1.98)	4.09	0.13
Ethyl formate	9.12 ± 0.82	1.7 (1.64-1.78)	2.37 (2.25-2.54)	3.06	0.22
Propyl formate	7.87 ± 1.70	1.69 (1.62-1.80)	2.45 (2.15-3.38)	2.66	0.10
Butyl formate	7.79 ± 0.76	1.54 (1.48-1.60)	2.25 (2.08-2.50)	4.50	0.10
Hexyl formate	7.52 ± 0.90	1.86 (1.77-2.00)	2.76 (2.47-3.29)	4.00	0.13
Heptyl formate	4.79 ± 0.55	3.17 (2.92-3.51)	5.88 (4.99-7.54)	4.56	0.33
EGDF	9.12 ± 0.81	2.99 (2.89-3.11)	4.14 (3.90-4.48)	1.98	0.96
Heterobicyclics					
Menthofuran	11.66 ± 1.72	3.62 (3.51-3.73)	4.66 (4.37-5.21)	2.36	0.49
Benzothiophene	4.83 ± 0.50	2.89 (2.71-3.10)	5.33 (4.70-6.42)	1.20	0.54
Dimethyl-coumarone	7.86 ± 0.49	2.98 (2.88-3.09)	4.35 (4.13-4.62)	4.94	0.55
Coumaran	3.14 ± 0.34	2.03 (1.84-2.26)	5.19 (4.22-7.05)	2.57	0.27
Acetate esters					
Propyl acetate	5.89 ± 1.22	4.31 (3.98-5.11)	7.11 (5.73-11.8)	0.24	0.88
Butyl acetate	7.83 ± 1.22	3.91 (3.73-4.21)	5.70 (5.04-7.16)	0.03	0.98
Pentyl acetate	8.04 ± 1.21	3.80 (3.65-4.05)	5.49 (4.91-6.72)	0.24	0.88
Hexyl acetate	6.15 ± 0.69	5.09 (4.75-5.41)	8.23 (7.52-9.38)	0.63	0.42

[a] Positive Control.

Table 5. Vapor toxicity of EGDF, heptyl formate & menthofuran with and without the synergistic effect of DEF and PBO to mosquitoes *Aedes aegypti* (L.)

Insecticides	Slope ± SE	LC_{50} mg/liter (95%CI)	LC_{90} mg/liter (95% CI)	$\chi 2$	P	Potency ratio [a]
EGDF	9.12 ± 0.81	2.99 (2.89-3.11)	4.14 (3.90-4.48)	1.98	0.96	
EGDF + DEF	6.36 ± 0.69	7.67 (7.23-8.08)	12.19 (11.19-13.80)	0.34	0.98	2.56 (2.39-2.73)
Heptyl formate	4.79 ± 0.55	3.17 (2.92-3.51)	5.88 (4.99-7.54)	4.56	0.33	
Heptyl formate + DEF	8.33 ± 0.82	4.29 (4.12-4.47)	6.12 (5.74-6.70)	2.20	0.69	1.35 (1.23-1.49)
Menthofuran	11.66 ± 1.72	3.62 (3.51-3.73)	4.66 (4.37-5.21)	2.37	0.49	
Menthofuran + PBO	8.47 ± 0.96	3.37 (3.24-3.52)	4.77 (4.39-5.42)	5.37	0.14	0.93 (0.89-0.98)

[a b] LC_{50} Chemical+synergist / LC_{50} Chemical alone.

Table 6. Body-weight corrected vapor toxicities of 15 novel, low molecular weight, volatile compounds and the organophosphate DDVP to mosquitoes *Aedes aegypti* (L.) and *Drosophila melanogaster* Meig.

Insecticide Families Insecticides	Mosquito LC_{50} mg/g of insect/liter (95%CI)	Drosophila LC_{50} mg/g of insect/liter (95%CI) [c]
Organophosphates		
DDVP[a]	1.7 (1.5-1.8)	3.7 (3.2-4.3)
Formates esters		
Methyl formate	88 (85.7-91.5)	824 (636.6-1,776)
Ethyl formate	112 (107.2-116.3)	550 (493.3-636.6)
Propyl formate	110 (105.8-117.6)	610 (593-626.6)
Butyl formate	100 (96.7-104.5)	304 (266.6-332)
Hexyl format	130 (115.6-130.7)	380 (356.6-400)
Heptyl formate	206 (190.8-229.4)	450 (420-475.3)
EGDF	194 (188.8-203.3)	834 (676.6-1,846)
Heterobicyclics		
Menthofuran	230 (229.4-243.7)	136 (120-150)
Benzothiophene	190 (177.1-202.6)	266 (236.6-293.3)
Dimethyl coumarone	194 (188.2-201.9)	654 (513.3-817.3)
Coumaran	132 (120.3-147.7)	490 (446.6-553.3)
Acetate esters		
Propyl acetate	282 (260.1-333.9)	683 (ND)[b]
Butyl acetate	256 (243.8-275.2)	607 (576.6-643.3)
Pentyl acetate	248 (238.5-264.7)	597 (550-660)
Hexyl acetate	333 (310.4-353.6)	553 (526.6-583.3)

[a] Positive control.
[b] Not determined.
[c] *Drosophila* data Scharf et al. 2006.

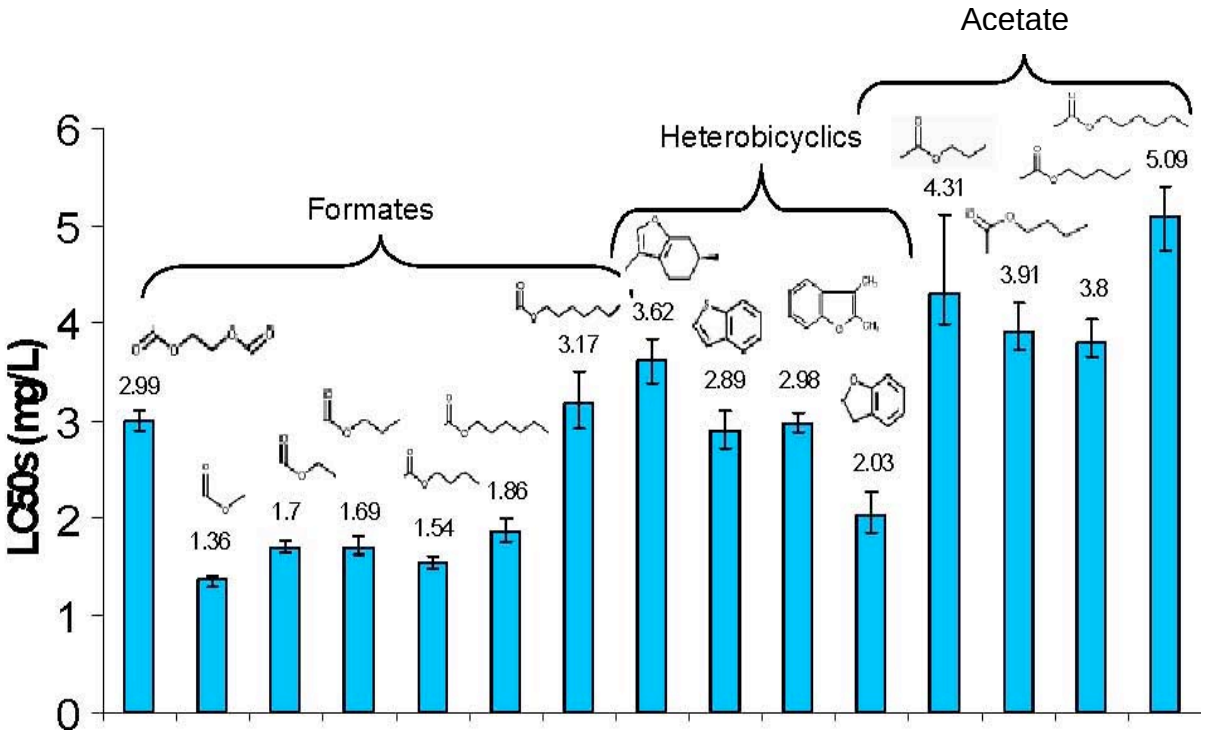

Figure 2. The LC_{50} values of mosquitoes *Aedes aegypti* (L.) when exposed on vapors of 15, novel, low molecular weight compounds.

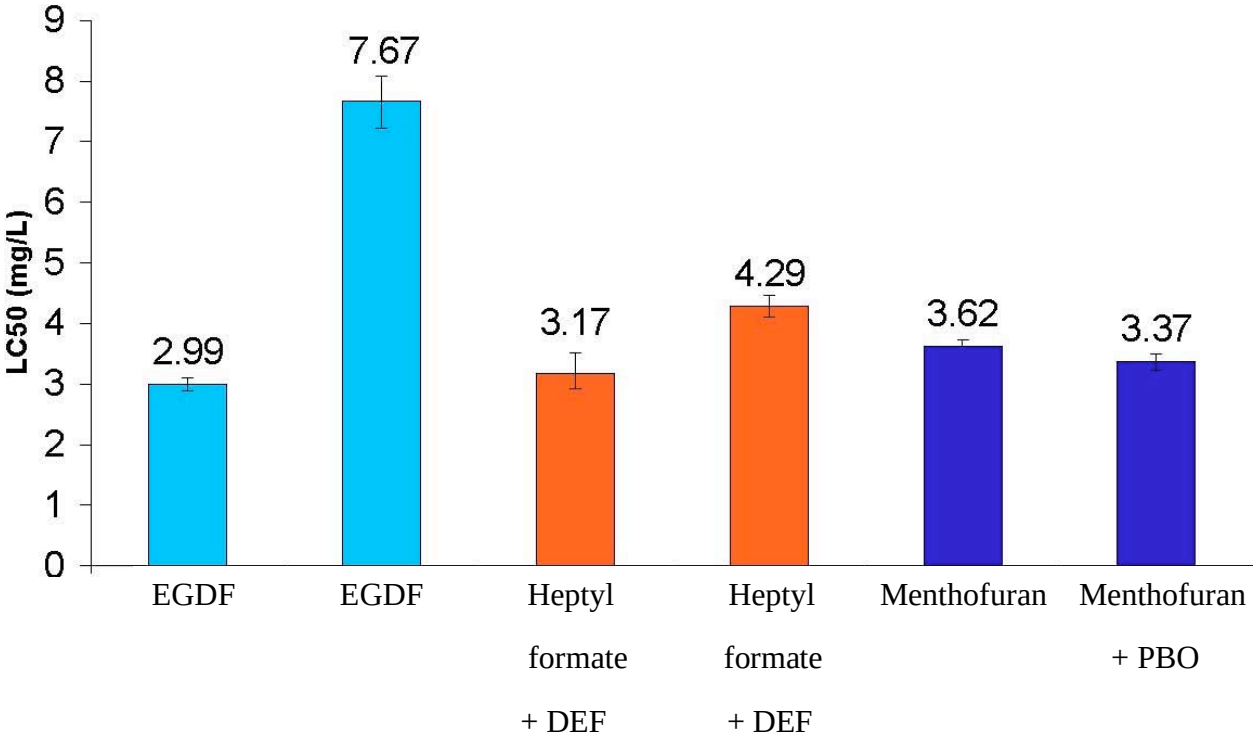

Figure 3. The LC_{50} values of mosquitoes *Aedes aegypti* (L.) when exposed on the vapors of EGDF, heptyl formate, and menthofuran with and without the synergistic effect of DEF and PBO.

III. Vapor Toxicity of Volatile Compounds to House Flies

Materials and Methods

Chemicals Three novel insecticides were tested; one heterobicyclic (menthofuran) and 2 formate esters [heptyl-formate and ethylene glycol diformate (EGDF)]. Dichlorvos (DDVP) was used as a positive control. The insecticide synergists SSS-tributyl-phosphorotrithioate (DEF) and piperonyl butoxide (PBO), which are esterase and cytochrome P450 inhibitors, respectively, were also used.

Ceramic Rods. Hydrophilic, porous, ceramic rods were used to provide controlled vapor release of the volatile compound heptyl formate. The rods were 7.5 cm in length and 1.3 cm in diameter. The porous size of the ceramic rods was 2.5 microns and 38% of each rod was void volume. In order to decrease insecticidal release rate, the rods were covered tightly with aluminum foil leaving one end exposed, prior to being treated with insecticide.

Insects. The Horse-Teaching-Unit (HTU) strain of *Musca domestica* (L.) was used. Three- to 5-d-old adult flies were aspirated from their cages and placed into plastic deli cups on ice until their activity was reduced. Ten females were used per replicate and a minimum of 300 flies was exposed to each insecticide.

Bioassay Main bioassay. Ten females were transferred into 125 ml vials covered with window screening (~1.55 mm mesh) to prevent insect escape while allowing for gas exchange. Insects were provided a cotton wick dipped in 10% sugar solution. Vials with flies were placed individually in 1-liter Mason jars along with a filter paper disk (55 mm in diameter) (Fig. 4). Insecticide solutions were applied to the filter paper and the jars were closed rapidly and tightly to prevent vapor escape. After a 24 h exposure, mortality was recorded.

DEF and PBO bioassay. This bioassay was similar to the one above with an extra step to expose mosquitoes DEF or PBO, which were applied to the mosquito vials before the transfer of the insects into them.

Controlled vapor release of heptyl formate. This bioassay was similar to the main bioassay but the insecticide (3.81 g of heptyl formate) was applied to the ceramic rods (Fig. 5). The experiment also included 3 other treatments: a blank control (rods only), 0.95 g of heptyl formate applied to filter paper, which is the estimated amount of heptyl formate released from the rod within 24 hrs, and 3.81 g of heptyl formate applied to filter paper. Every day for 9 days, the treated rod or filter paper was transferred to a new jar with fresh flies. Mortality was determined after 24 hrs exposure to the treatments.

Data Analysis. Where appropriate mortality adjusted using the Abbott's formula and LC_{50} and LC_{90} were estimated using probit analysis and compared using 95% confidence limits. Average insect weight was used to adjust dose from mg/liter of air to mg/g of insect body weight/liter of air. Body-weight corrected LC_{50} of each insecticide for house flies and *Drosophila* were calculated. PoloPlus 2.0 statistical software was used to calculate the potency ratios of the LC50s with and without the DEF or PBO. Also, SNK (Student-Newman-Keuls) test was performed to determine the day when significant decrease in house fly mortality for the rod (3.81 g) treatment was seen (SAS Institute 2003).

Results

Toxicity Evaluation of Novel Compounds DDVP was 25X more toxic than the second best compound, the heterobicyclic menthofuran (LC_{50} = 3.70 mg/liter) (Table 7). EGDF was the next most toxic compound (LC_{50} = 9.27 mg/liter) and heptyl formate was the least toxic compound (LC_{50} = 32.62 mg/liter, respectively). DEF decreased the toxicity of heptyl formate and EGDF. The toxicity of menthofuran increased by 1.5X when synergized with PBO (Fig. 6).

Toxicities of Novel Compounds to House Flies and *Drosophila*. There were significant differences among the toxicities of the compounds to house flies and *Drosophila* (Table 8). Overall, all the compounds were more toxic to house flies than *Drosophila*. DDVP was the most toxic insecticide for both insects and was 5.2X more toxic to house flies than *Drosophila*. On average the volatile compounds were approximately by 10X more toxic to house flies than *Drosophila*. Menthofuran was the most toxic novel compound against both insects. Heptyl formate was more toxic to *Drosophila* than EGDF and less toxic than EGDF to house flies.

Controlled Release of Heptyl Formate. The filter paper treated with 0.95 g of heptyl formate caused 100% fly mortality for the first day only (Table 9). The filter paper treated with 3.81 g of heptyl formate caused fly mortality for 3 days only. The rod embedded with 3.81 g of heptyl formate caused fly mortality throughout the duration of the experiment, but fly mortality on the 9th day was significantly lower than that at the start of the experiment.

Figure 4. Mason jar set up to test Heptyl formate for control of flies.

Figure 5. Mason jar set up to test controlled release of volatile for control of flies.

Table 7. Vapor toxicity of EGDF, heptyl formate, and menthofuran with and without the synergistic effect of DEF and PBO and the organophosphate DDVP to house flies *Musca domestica* (L.)[b]

Insecticide	Slope	LC_{50} mg/liter	LC_{90} mg/liter	χ	P	Potency ratio ± SE (95%CI)
DDVPa	9.6 ± 1.0	0.148 (0.14-0.15)	0.202 (0.19-0.22)	2.35	0.67	-
EGDF	7.4 ± 0.8	9.27 (8.75-9.75)	13.81 (12.81-15.36)	9.76	0.14	
EGDF + DEF	7.9 ± 0.7	18.56 (17.76-19.33)	26.88 (25.34-29.02)	8.10	0.23	2 (1.87-2.14)
Heptyl formate	4.1 ± 0.6	32.62 (30.21-35.44)	66.89 (55.88-91.58)	3.87	0.79	
Heptyl formate + DEF	6.9 ± 1.2	48.70 (46.20-51.63)	74.45 (65.94-94.29)	3.76	0.29	1.5 (1.36-1.64)
Menthofuran	10.6 ± 1.1	3.70 (3.58-3.83)	4.88 (4.61-5.32)	4.29	0.12	
Menthofuran + PBO	4.8 ± 1.2	2.43 (1.85-2.69)	4.49 (3.90-6.59)	0.49	0.48	0.65 (0.56-0.76)

[a] Positive control.
[b] LC_{50} Chemical+synergist / LC_{50} Chemical alone.

Table 8. Body-weight corrected vapor toxicities of EGDF, heptyl formate, menthofuran and the organophosphate DDVP to house flies *Musca domestica* (L.) and *Drosophila melanogaster* Meig.

Treatment	Housefly LC_{50} (mg/g of insect/liter)	Drosophila LC_{50} (mg/g of insect/liter)[b]
DDVP[a]	0.7 (0.67-0.72)	3.7 (3.2-4.3)
EGDF	44 (41.15-45.86)	834 (676.6-1,846)
Heptyl formate	153 (142.1-166.7)	450 (420-475)
Menthofuran	17.4 (16.83-18)	136 (120-150)

[a] Positive control

[b] *Drosophila* data Scharf et al. 2006

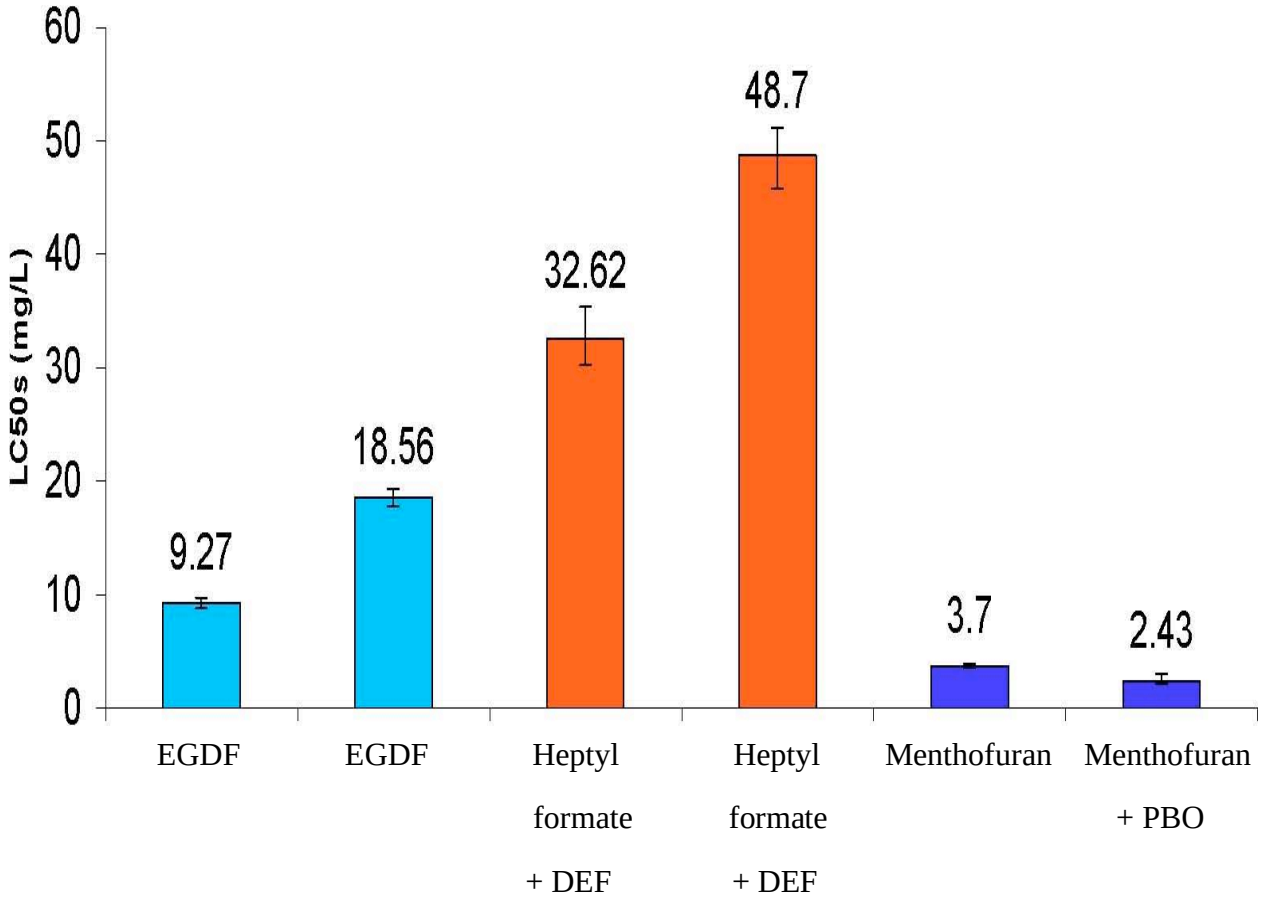

Figure 6. Vapor toxicity of EGDF, heptyl formate, and menthofuran with and without the synergistic effect of DEF and PBO to the house flies *Musca domestica* (L.).

Table 9. Percent mortality of controlled vapor release of heptyl formate on house flies Musca domestica (L.) over 9 days among 3 different treatments and a blank control of Heptyl Formate on House Flies

Time (days)	Percent Mortality	Control Filter Paper (3.81g)	Filter Paper (0.95 g)	Ceramic Rod (3.81 g)
Day 1	0	100	100	100a
Day 2	0	100	0	100a
Day 3	0	100	0	98 ± 2a
Day 4	0	0	0	96 ± 4a
Day 5	0	0	0	88 ± 5.8ab
Day 6	0	0	0	84 ± 6.8ab
Day 7	0	0	0	82 ± 5.8ab
Day 8	0	0	0	74 ± 10.3ab
Day 9	0	0	0	64 ± 14.7b

Percentages followed by the same letter are not significantly different (SNK test, SAS Institute, 2003).

IV. Insecticide-Impregnated Cords for House Fly Control

Materials and Methods

Insects. The Horse Teaching Unit (HTU) strain of house flies, *M. domestica* L., was used for all experiments**.** Adult house flies (3-5 d old) were aspirated from the screened rearing cages, and placed into a 5°C environment for 5 min to subdue activity, except for flies used for field cage assays, which were not anesthetized. Flies were sexed, and counted.

Laboratory Arenas. Arenas (31 x 25 x 21 cm) were constructed using PVC pipe (1.27 cm [0.5 in]) (Fig. 7a). Rubber bands were used to establish individual treatment positions to which the treatment cords were attached and arenas were enclosed with a transparent plastic bag.

Cord Attractiveness Bioassay. Eight cords were evaluated: nylon (Braided, Multi-Purpose Braid 75 lb. load limit, Wellington Cordage LLC, Madison, GA), polypropylene (Braided, Multi-Purpose Rope – 56 lb. load limit, Wellington Cordage LLC, Madison, GA), cotton (Braided, Multi-Purpose Sash Cord – 28 lb. load limit, Wellington Cordage LLC, Madison, GA), cotton wick (Sterilized roll, #200209, Richmond Dental Company, Charlotte, NC), manila (Twisted, Natural Rope – 108 lb. load limit, Wellington Cordage LLC, Madison, GA), wool (Twisted, Natural Cord, Wooded Hamlet Designs, Greencastle, PA), leather (Tan laces, #6192, Rothco, Ronkonkoma, NY), and parachute cord (550 test, white, purchased locally from M & C Army Surplus Store, Gainesville, FL). Fifty female flies were released into each arenas and 10% sugar water was provided *ad libitum*. Number of flies resting on cords was counted every 10 min for 2 hr.

Impregnated-Cord Laboratory Bioassays. Bioassay set up was similar to the one described above. Cotton, manila, wool, polypropylene, and nylon cords were impregnated with a 0.1% fipronil or a 0.6% indoxacarb solution by dipping for ~2 sec followed by drying in a fume hood. Mortality was recorded until it reached at least 80%.

Impregnated-Cord Field Cage Bioassay. Cages (1.8 x 3.7 x 1.8 m) were constructed from PVC pipe (2.54 cm [1 in] diam.) and enclosed with mesh screening. Black plastic sheeting (6 mil) was used to line the floor. A sampling stage was placed in the center of the cage (Fig.7b) with two 1-L chick waterers, one filled with 10% sugar water and the other with tap water, and a 60-ml plastic cup with 8 g of previously used larval house fly medium covered with a paper towel. Treatments consisted of two long (0.9 m) and eight short (0.6 m) lengths of 0.1% fipronil- and 1.2% indoxacarb-impregnated wool cords treated as in the laboratory experiments. Flies (27.5 – 35 ml at 9.8 ± 1.8 flies/ml) were released into each cage. After a 1-h acclimation period, pre-treatment fly counts were taken. Before fly counts were taken, the flies were disturbed from their resting positions dead flies were collected from the floor. Four consecutive fly counts were then taken from the outside of the cage of all flies on the sampling stage, chick waterers, and plastic cup attractant. Treatments were then hung vertically from the mesh ceiling using paper clips in specific locations (Fig. 7c) and post-treatment fly counts were taken at 24 and 48 h. After the initial 48 h evaluation, treatments were aged in the elements for four weeks, at which point residual effectiveness was re-evaluated. Three replicates were performed at each treatment age (0 and 4 wk).

Statistical Analysis. For the cord attractiveness experiments, the mean number of flies/cord was analyzed using a one-way analysis of variance followed by means comparisons. For the laboratory insecticide-impregnated cord experiments, a two-way analysis of variance was

performed on the data and LT_{50} values were estimated. For the field cage experiments, percent fly reductions and mortality data were then analyzed for each treatment age (0 and 4 wk)

Results

In the laboratory studies, flies were more attracted to the manila cord than any other cord (Fig. 8). No significant differences were seen between the other natural cords or between the synthetic cords, but all synthetic cords attracted significantly less flies than the natural cords, and the animal-based cords were more attractive than the plant-based cords. At the 24 h (fipronil) and 48 h (indoxacarb) recordings, all impregnated-cords had significantly higher mortality than the controls (Fig. 9). House flies suffered significantly higher mortality when exposed to the fipronil-impregnated wool cord than any other fipronil-impregnated cord at (93%), and the indoxacarb-impregnated wool cord caused significantly higher mortality (85%) than any of the other cords except for the cotton cord. Nylon cord with either insecticide caused the lowest mortalities. The fipronil-impregnated cords had lower LT_{50} values than the indoxacarb-impregnated cords (Table 10). All indoxacarb-impregnated cords had LT_{50} values above 32 h. In the field cage experiments, all treatments had over 45% fly reductions by 24 h and 95% fly count reductions by 48 h, independent of the treatment age (Table 11). Fipronil treatments had significantly more dead flies than the indoxacarb treatments with fresh cords and with aged cords at 24 h.

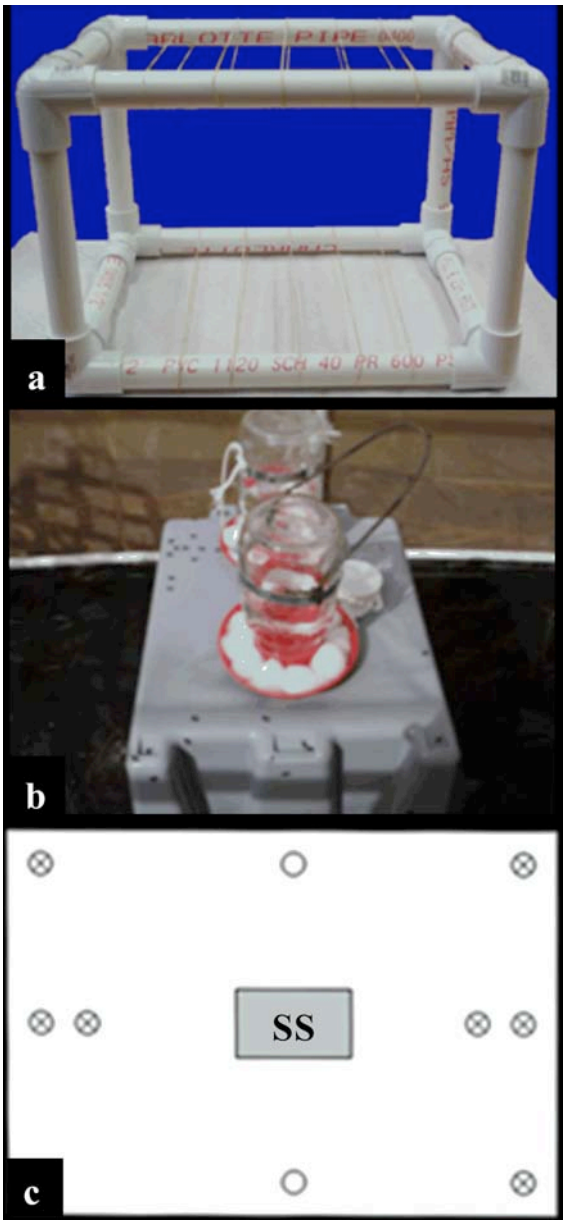

Fig. 7. a) Laboratory arena constructed of PVC pipe. Cords were suspended between the rubber bands using paper clips; b) Sampling stage used in field cage experiments with approximately 30 flies. Chick feeders with either 10% sugar water or tap water and a plastic cup containing previously used larval medium was used as sustenance and attraction; c) Bait-treated cord placement in relation to sampling stage ("SS"; centered) in the field cage bioassay. Crossed circles were short cords (0.6 m) and empty circles were long cords (0.9 m).

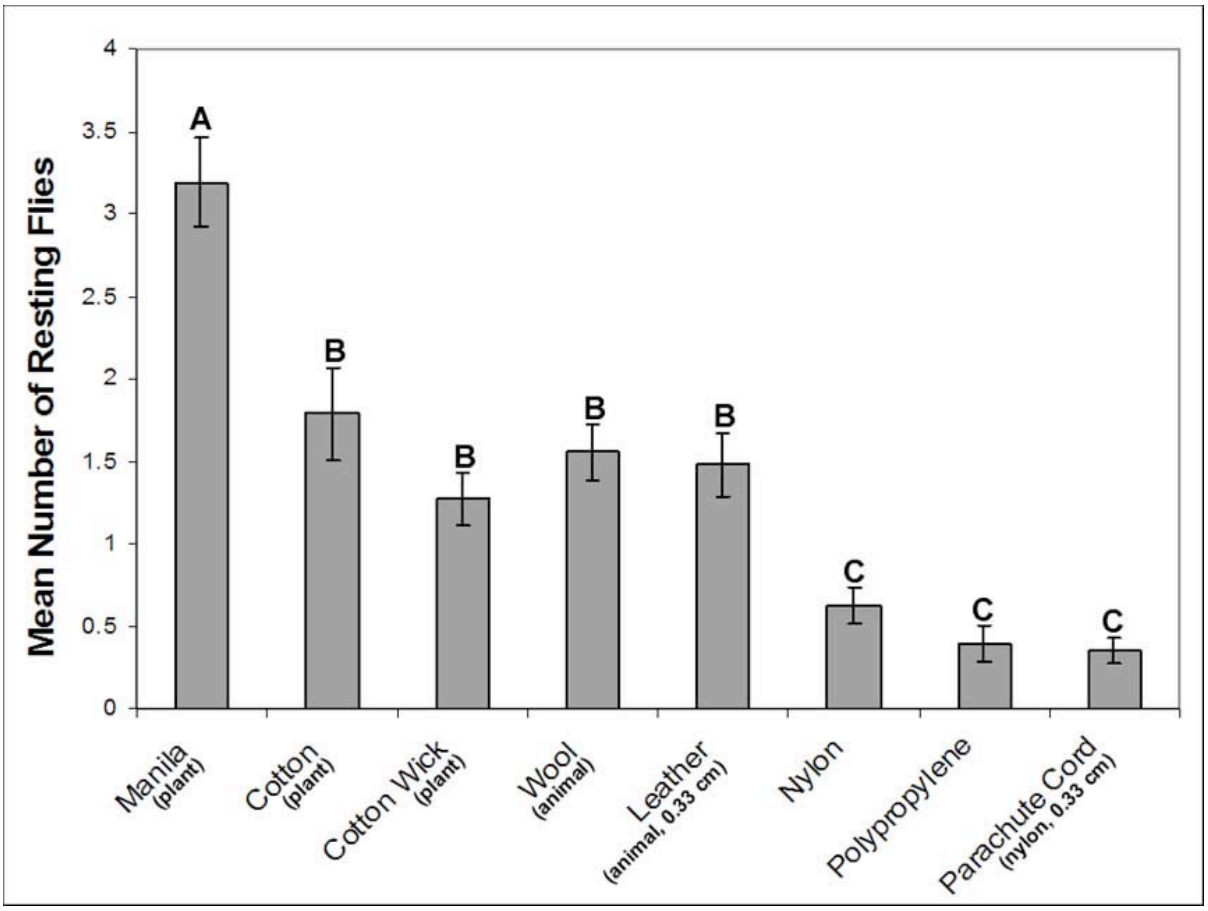

Fig. 8. Attraction of female house flies to various natural and synthetic cords.

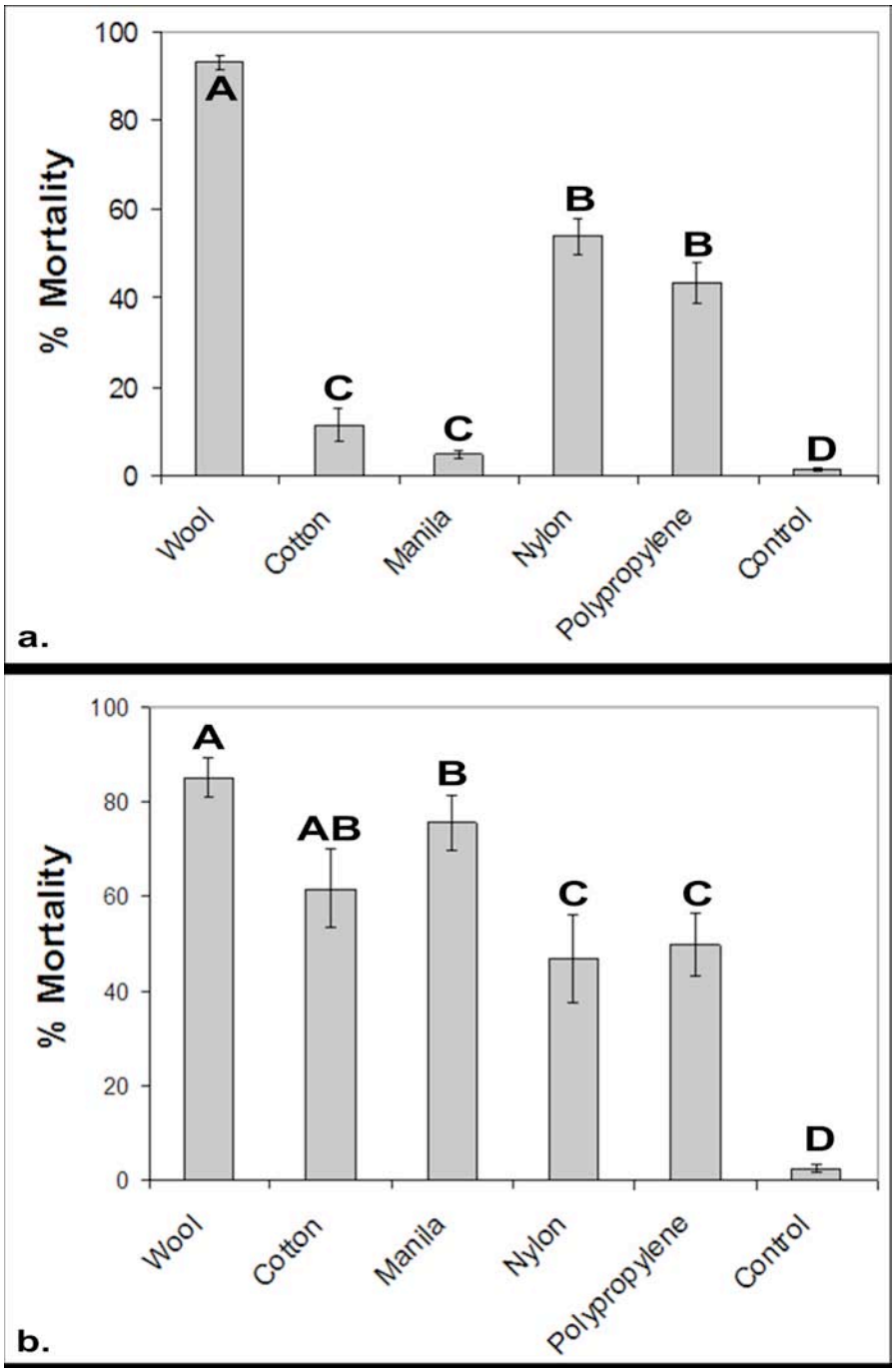

Fig. 9. Female house fly mortality exposed to various natural and synthetic cords treated with 0.1% fipronil for 24 h (a.) and 0.6% indoxacarb for 48 h (b.). Data were corrected by Abbott's formula and arcsine square root-transformed prior to analysis. Means were separated using the SNK method (α=0.05). Raw data is plotted above.

Table 10. LT_{50} values (h) of female house flies exposed to various cords impregnated with a 0.1% fipronil and 0.6% indoxacarb solution.

Treatment	Cord	$n^{\dagger}$	Slope ± SE[§]	LT_{50} (95% CL)[§]	χ^2	P
Fipronil	Cotton	2750	9.52 ± 0.36b	39.7 (39.2-40.2)e	9.060	0.1067
	Manila	1000	12.98 ± 0.83a	35.0 (34.5-35.6)d	1.213	0.5445
	Wool	1250	5.32 ± 0.29c	12.9 (12.3-13.4)a	0.650	0.4200
	Polypro	2500	4.65 ± 0.29d	26.2 (25.6-27.0)c	3.643	0.7249
	Nylon	1500	3.68 ± 0.25e	23.0 (21.2-24.6)b	1.455	0.6927
Indoxacarb	Cotton	2248	4.04 ± 0.13c	52.2 (50.3-54.3)c	1.3494	0.5093
	Manila	1659	5.10 ± 0.74b	36.2 (32.3-38.7)ab	3.4030	0.3336
	Wool	4250	6.44 ± 0.16a	32.6 (32.0-33.1)a	8.6295	0.2804
	Polypro	2250	3.11 ± 0.20d	52.2 (49.7-54.5)c	0.6717	0.7147
	Nylon	2000	6.57 ± 0.54a	39.2 (37.2-40.8)b	2.9321	0.2308

[†] Total number of trials (Probit [SAS Institute 2002]).
[§] Time in hours (h). Means within a column, in the same treatment group, followed by the same letter are not significantly different based on non-overlap of 95% confidence intervals.

Table 11. Cumulative number of dead flies and percent fly count reduction in relation to pre-treatment fly counts of house flies exposed to 0.1% fipronil- and 1.2% indoxacarb-impregnated cords in field cages.

		% Fly Count Reduction ± SEM[†‡]		# of Dead Flies§[‡]	
Treatment Age	Treatment	24 h	48 h	24 h	48 h
0 Weeks	Fipronil	80.22 ± 13.10a	98.66 ± 1.34a	83 ± 1.0a	95.33 ± 3.38a
	Indoxacarb	57.39 ± 6.92a	97.21 ± 1.43a	30.33 ± 4.41b	59.67 ± 2.03b
4 Weeks	Fipronil	59.25 ± 22.10a	87.43 ± 12.57a	53.0 ± 7.77a	79.33 ± 7.22a
	Indoxacarb	64.39 ± 5.89a	87.72 ± 7.34a	30.33 ± 4.67b	62.00 ± 8.14a

[†] Reduction based off of control. Data was rank-transformed prior to analysis.
§ Cumulative mean number of flies recovered from cage floor.
[‡] Means in a column, within the same treatment age, followed by the same letter are not significantly different ($P > 0.05$; Student's T test)

V. New Imidacloprid bait for House Fly Control

Materials and Methods

Insects and laboratory arenas and field cages were as described in previous section.

Laboratory Fly Bait Comparisons. Three fly baits, 2 dry scatter baits (Golden Malrin®, dose: 0.23 g/0.9 m^2; Maxforce® Granular fly bait, 30.17 g/0.9 m^2, both sprinkled on petri dishes), and 1 sprayable bait (Maxforce® Fly Spot bait, dose: 0.45 g/0.9 m^2, sprayed on petri dishes), were placed in the center of the laboratory arenas. Untreated cords hung in the arenas served only as resting positions for the flies. Groups of 50 female flies were placed within each arena and mortality was recorded at 1, 3, 5, and 24 h.

Field Fly Bait Comparisons. The two imidacloprid baits were mixed with tap water and applied to plastic lattice squares (0.19 m^2) and evaluated in the field cages after drying. Data collection and other procedures for filed cage tests were as described in previous section except that aging in the elements was for two weeks only.

Bait-Treated Cords. Treatment consisted of different cords as described in previous section (15.2 cm length, 0.6 cm diam.) impregnated with a 2.5% solution of imidacloprid sprayable bait. Groups of 60 female flies were released per arena, and morbidity (knockdown) was recorded at 2-5 h post-treatment and mortality was recorded at 24, 48, and 72 h.

Field Test of Bait-Treated Cords. Procedures were similar to those described in previous section for field cage tests. Treatments consisted of two long (0.9 m) and eight short (0.6 m) lengths of imidacloprid-impregnated wool cords. Cords were hung vertically from the mesh ceiling using paper clips in specific locations, which remained constant throughout the experiment (Fig. 1c). Post-treatment sampling counts were done at 24 and 48 hrs for both freshly prepared cords and again with the cords aged in the elements for four weeks.

Data Analysis. All analyses were done using a one-way analysis of variance. Percent morbidity and mortality were arcsine square root-transformed before analysis. Means for all analyses were separated using the Student's T test or the Student Newman Kuels (SNK) method ($\alpha = 0.05$).

Results

Fly Bait Comparisons. The imidacloprid granular and the imidacloprid sprayable baits had higher fly mortality than the methomyl granular fly bait at 3 h, but by 24 h the methomyl granular bait had the highest overall mortality (Fig. 10). All treatments were significantly different than the control fly mortality at all observations $\geq$ 3 h. In the field cage experiments, no differences were seen between treatments with fresh or aged cords (Table 12), with >35% fly count reductions at 24 h and >70% by 48 h with fresh cords. Fly count reductions did not exceed 8% with aged cords for either treatment.

Bait-Treated Cords. Morbidity increased on a time-dependent basis until approximately 3-4 hours post-treatment, but some flies recovered from being knocked down by the imidacloprid bait-treated cords (Fig. 11). All treated cords caused significantly more mortality than the control cords at every 24 h recording (Fig. 12). The imidacloprid bait-treated wool cord caused the highest overall fly mortality (74%), whereas all other cords resulted in <60% house fly mortalities. In the field cages, the imidacloprid bait-treated cords caused >87% fly reductions by 24 h with fresh and aged cords (Table 13). The number of dead flies collected was

significantly different than the control at 24 and 48 h for both fresh and aged cords, except for the 48 h recording with the aged cords.

Table 12. Number of dead and percent fly count reduction in relation to control fly counts of house flies exposed to imidacloprid bait-treated lattice squares in field cages.

		% Fly Count Reduction ± SEM‡		# of Dead Flies†‡	
Treatment Age	Treatment	1 h	24 h	1 h	24 h
0 Weeks	Imidacloprid granular bait	47.1 ± 6.3a	70.9 ± 4.4a	36.0 ± 10.0a	117.0 ± 9.5a
	Imidacloprid sprayable bait	36.6 ± 20.5a	80.2 ± 4.7a	36.3 ± 2.0a	113.0 ± 10.1a
	Control			0.3 ± 0.3b	1.7 ± 0.7b
2 Weeks	Imidacloprid granular bait	0.8 ± 8.2a	-3.8 ± 20.1a	1.0 ± 0.6a	19.7 ± 11.2a
	Imidacloprid sprayable bait	7.6 ± 10.8a	6.3 ± 19.5a	0.7 ± 0.7a	14.0 ± 11.6a
	Control			0.7 ± 0.7a	10.3 ± 5.6a

† Mean number of individuals recovered from cage floor.
‡ Means in a column, within the same treatment age, followed by the same letter are not significantly different ($P > 0.05$; Student's T test or Student-Newman Kuels Method)

Table 13. Number of dead and percent fly count reduction in relation to control fly counts of house flies exposed to imidacloprid bait-treated cords in field cages.

		% Fly Count Reduction ± SEM		# of Dead Flies†	
Treatment Age	Treatment	24 h	48 h	24 h	48 h
0 Weeks	Bait-Treated Cords	90.4 ± 4.1	96.8 ± 3.2	97.7 ± 17.2a	114.3 ± 19.8a
	Control			12.7 ± 4.6b	19.7 ± 7.7b
4 Weeks	Bait-Treated Cords	87.9 ± 0.9	82.4 ± 14.7	50.7 ± 14.0a	69.3 ± 23.7a
	Control			8.7 ± 3.8b	13. ± 4.7a

† Mean number of individuals recovered from cage floor. Means in a column, within the same treatment age, followed by the same letter are not significantly different ($P > 0.05$; Student's T test)

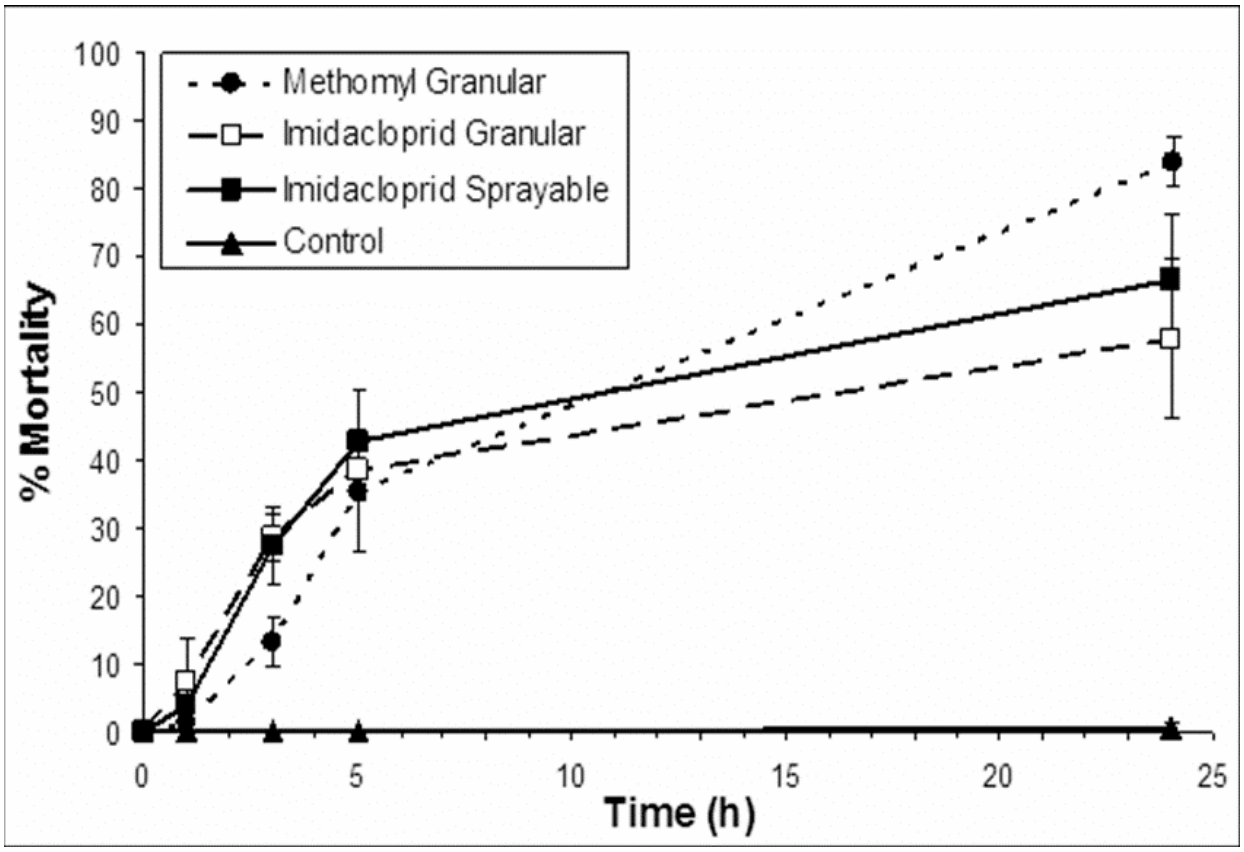

Figure 10. Mortality of female house flies exposed to imidacloprid and methomyl granular scatter baits and a sprayable imidacloprid bait.

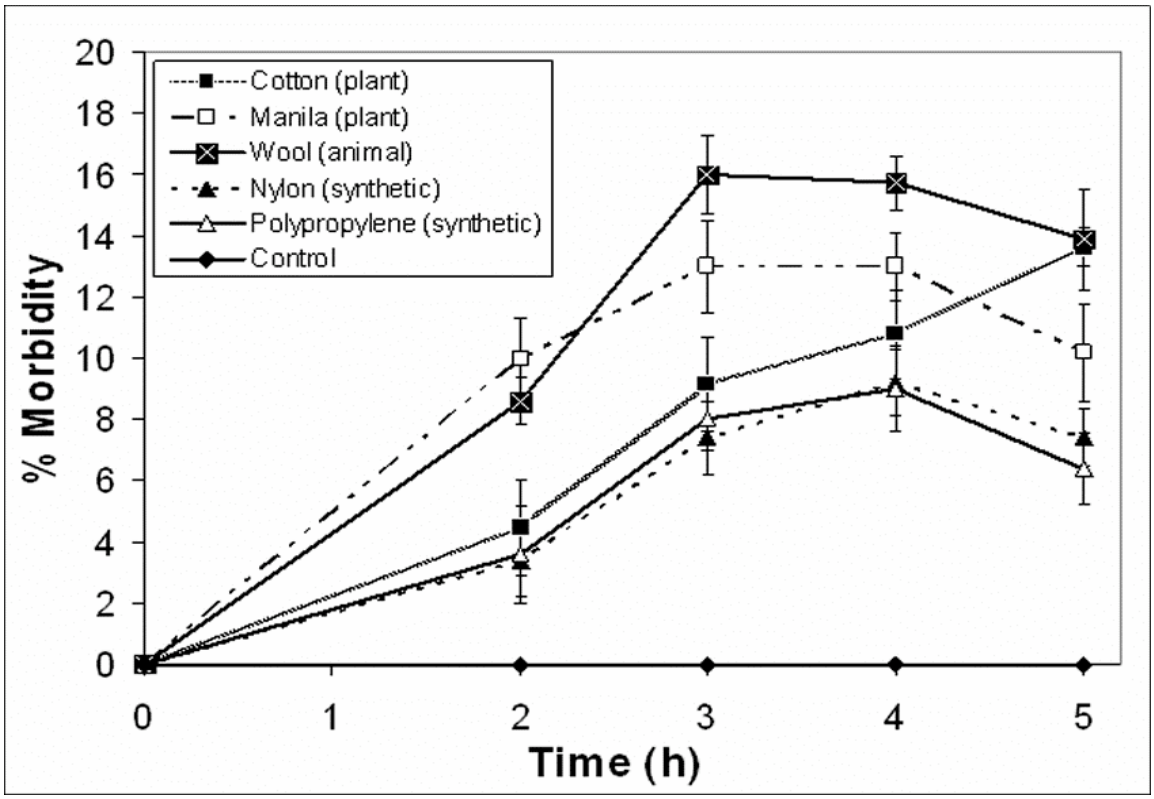

Figure 11. Morbidity (knockdown) of female house flies exposed to natural and synthetic cords dipped in a 2.5% solution of imidacloprid sprayable bait.

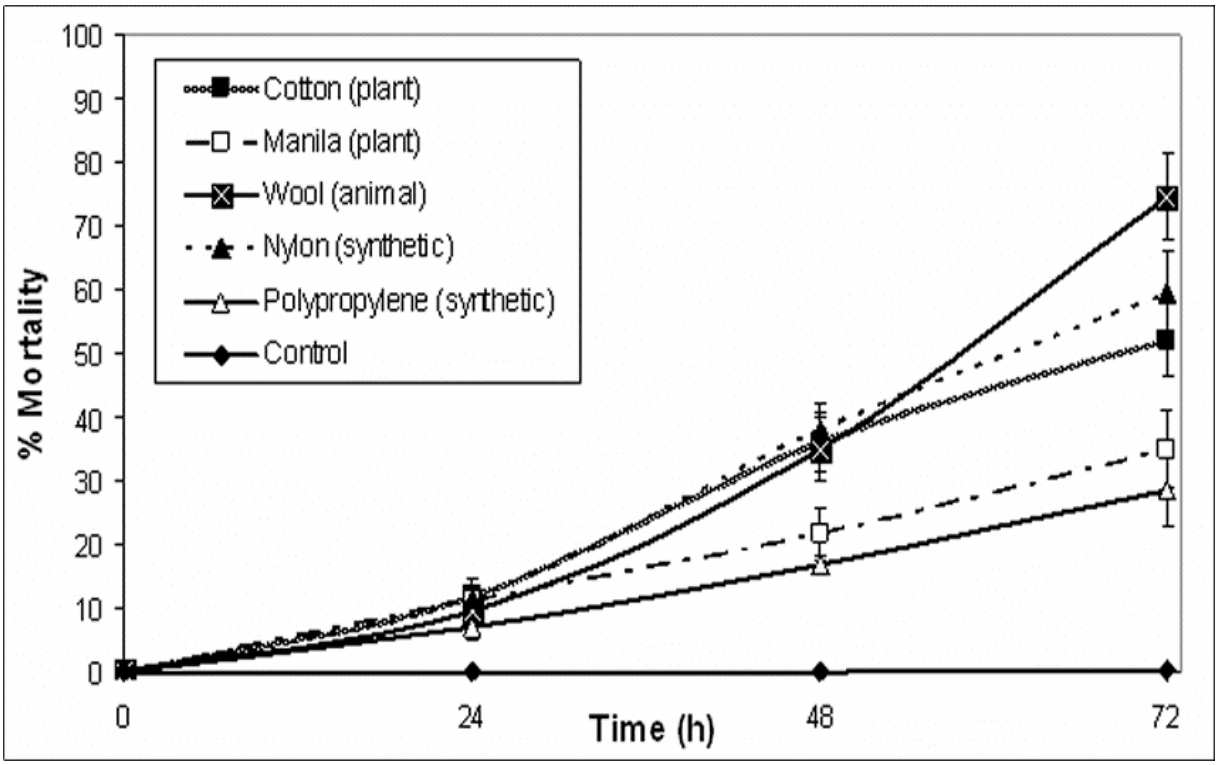

Figure 12 Mortality of female house flies exposed to natural and synthetic cords dipped in a 2.5% solution of imidacloprid sprayable bait.

VI. Armed Forces Pest Management Board National Stock Number Request.

Based on the results presented in the previous sections, an Issue Paper was submitted to the Pesticide Committee of the Armed Forces Pest Management Board (AFPMB) recommending that a National Stock Number (NSN) be assigned to the imidacloprid sprayable bait. During the Aug 2007 AFPMB meeting, HM1 Joe Diclaro made a presentation to the Pesticide Committee, which then voted to accept the recommendation on the Issue Paper. The matter was brought to the AFPMB council meeting and approved unanimously. The Issue Paper was as follows:

Issue Paper
for
Armed Forces Pest Management Board Pesticide and Contingency Advisory Committees
Date: August 13, 2007

Subject:

New sprayable/paintable fly bait for use indoors and outdoors (Maxforce Fly Spot Bait)

Committee(s)

Primary: Pesticide Committee; Secondary: Contingency Advisory Committee.

Background

Filth flies are a constant problem in military facilities, both in the US and in deployment conditions. Large numbers of flies are produced on several substrates and invade food preparation facilities, mess halls, field hospitals and other facilities where these flies can serve as vectors of disease organisms and be great annoyance. Currently the AFPMB has stock numbered several traps and pesticides for control of flies but only 3 products are listed in the "DOD CONTINGENCY PESTICIDES" (QuikStrike fly strips – NSN 6840-01-467-0994; MaxForce Granular Fly Bait – NSN 6840-01-518-5807, and Blue/Streak / Golden Malrin / Stimukill Granular Bait – NSN 6840-01-183-7244). Each of these products has limitations for military use including odor, staining, or indoor use restrictions.

Maxforce Fly spot bait is a wettable granule that is mixed with water and sprayed or painted on surfaces. The product dries to form a clear film containing a combination of attractants including the fly pheromone Z-9-Tricosene. Once applied, the product attracts flies that acquire lethal doses of the active ingredient (imidacloprid) by ingestion and/or contact.

Discussion

A new fly bait formulation of the pesticide imidacloprid offers several advantages over the previously available MaxForce Granular Fly Bait (NSN 6840-01-518-5807) and other baits

containing methomyl as active ingredient. MaxForce Spot Fly bait can be applied both as a spray or painted on surfaces. This product dries clear without staining the surfaces so there is interference with camouflage or substrate appearance. The label allows for application within serving areas of food establishments when food is not present, as well as outside living quarters and other structures.

The sprayable bait can also be used to impregnate cords, which have been used extensively in the past to control flies. Wool and other cords treated with this product showed good efficacy when treated with the imidacloprid sprayable bait. This mode of application can provide more flexibility in the control of filth flies.

Alternatives

No products currently assigned NSN have the desirable characteristics in the proposed product.

Recommendation(s)

Assign NSN to Maxforce Spot Fly Bait.

Jeffrey C. Hertz, LTJG (FMF) USN

Joseph W. Diclaro, II, HM1 (SCW) USN

University of Florida
Entomology & Nematology Dept.

VII. New Fly Control Methods

Materials and methods

Indoxacarb bait for fly control. The experimental setup consisted of cages (31 x 25 x 21 cm) constructed using PVC pipe (1.27 cm diam.) with supply of 10% sugar water (*ad libitum*) and 5 g of Tast-E-Bait®. Four moisture treatments, a dry control, and an untreated control (with Tast-E-Bait without any active ingredient) were used. The treatments consisted of dry Tast-E-Bait® containing 0.22% Indoxacarb (Advion Mole cricket bait - AMC Bait) and water, added to the bait to obtain Tast-E-Bait® to water ratios of: 1:1, 1:2, 1:3, 1:4. Fifty lab-reared female house flies were released into each arena. Fly mortality was assessed at 1, 3, 24 and 48 hours after exposure of the flies to the bait.

Stability of Maxforce fly spot bait at high temperatures. Maxforce® Fly Spot Bait was diluted following label instructions and painted on 24 2"X3" pieces of plywood. Bait-treated wood block were placed in oven at 60°C for varying up to 10 days. Wood blocks were removed at 1, 4, 5, and 10 days and tested against flies together with blocks that were never heat-treated and a untreated control that received no insecticidal bait. Wood blocks were placed in arenas as described above with 30 female house flies (Fig. 13). Mortality was evaluated at 1, 16, 24, and 48 hours.

Combination of traps and treated cord for fly control. This experiment tested different combinations of a commercial fly trap and a imidacloprid fly spot bait. Experiments were conducted in field fly cages described previously for bait-treated cords and other experiments. Six treatments were used: 1) untreated Control, (no bait, trap or cord), 2) a bait-treated wool cord treated with Maxforce fly spot bait formed into a circular halo, 3) a Trap n' Toss™ (Farnam Companies, Inc., Phoenix, AZ) fly trap with the fly attractant provided by the manufacturer, 4) a trap as in #3 wrapped with a bait-treated halo as in #2, 5) a trap + attractant as in #3 wrapped with a untreated wool cord halo, 6) a trap as in #3 but without any fly attractant but still wrapped with a bait-treated halo as in #2. Treatments were added to the field cages and 300 to 500 flies were added to the cages. Fly mortality was evaluated after 1, 24, and 48 hours.

Results

Indoxacarb bait for fly control. The Advion mole cricket bait formulated with Tast-E bait was very effective on killing house flies. Except for the dry bait, which caused only 30% fly mortality after 48 h, all other bait treatments reached close to 100% fly mortality in the same period (Fig. 14). The mixture 1:2 (bait:water) seems to be best because it uses less water and the flies die a faster than some of the other treatments.

Stability of Maxforce fly spot bait at high temperatures. Maxforce® Fly Spot Bait when applied to wood blocks and heat-treated at 60°C was very stable for up to 10 days (Fig. 15). Fly mortality was not significantly different for all treatments that received the fly bait independent of the heat-treatment length. Also, speed of kill did not seem to be affected by heat-treatment of the bait-treated wood block.

Combination of traps and treated cord for fly control. Treatments that received the treated wool cord hale caused between 85 and 95% fly mortality, and were not significantly different from each other. Fly mortalities in treatments with fly trap but no treated wool cord were only close to 30% and significantly lower that treatments with the treated halo (Fig 16). Fly

mortality in the control cages was lower than 10%. Results demonstrated that the bait-treated wool cord halo alone can cause high fly mortality and that its performance is not improved by the presence of the fly traps, independent of the presence of the trap fly attractant.

Figure 13. Cages used for testing oven-aged MaxForce spot fly bait applied to wood blocks.

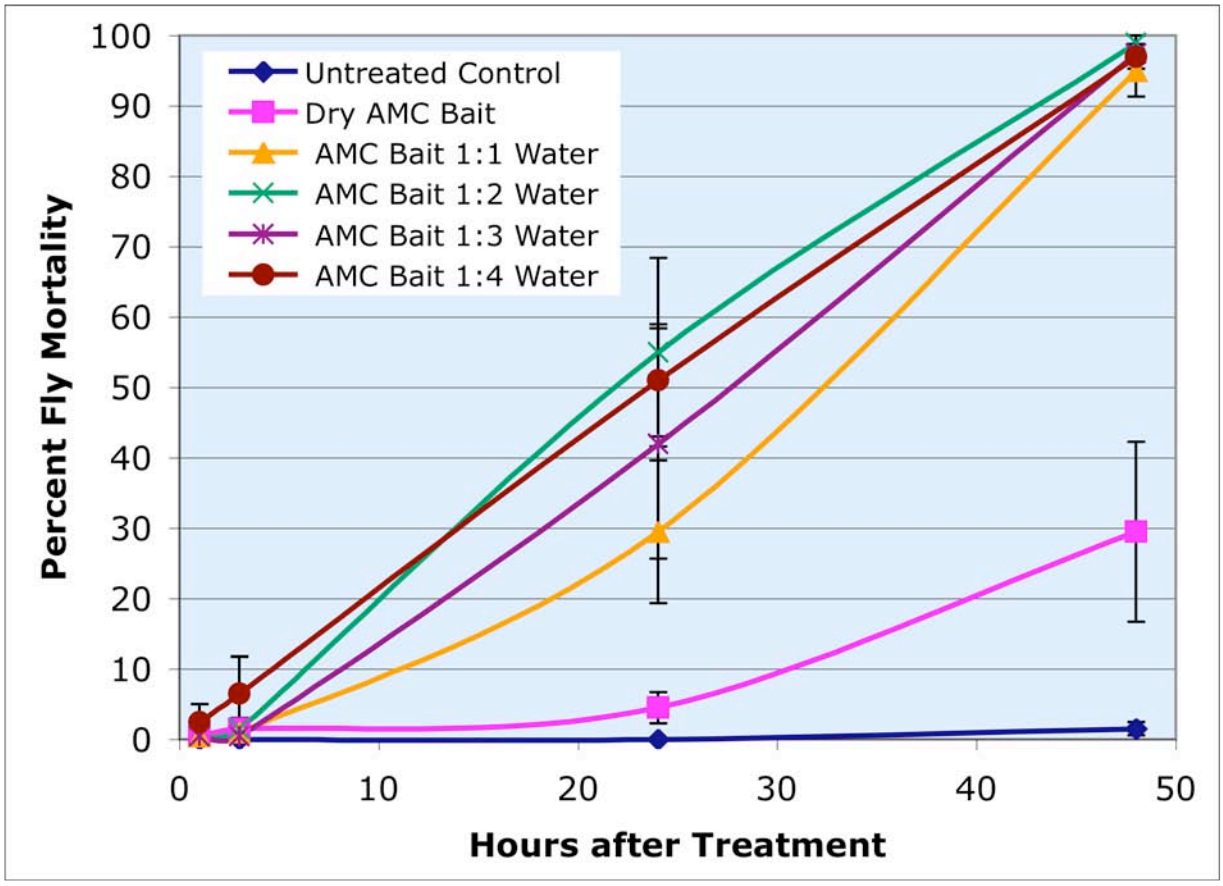

Figure 14. Percent mortality of house flies exposed to Advion mole cricket bait, which is a formulation of the carrier Tast-E with indoxacarb as the active ingredient, with different ratios of water added.

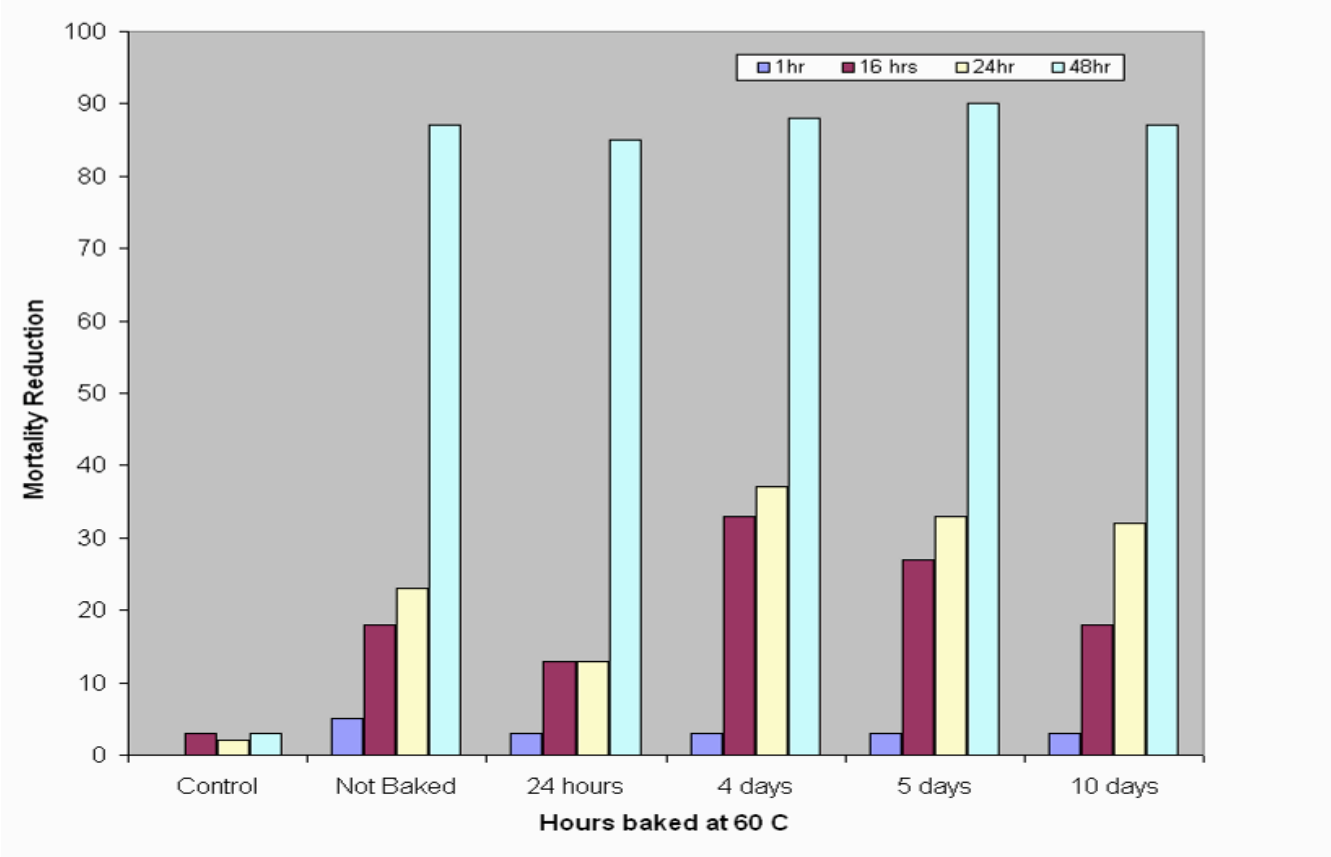

Figure 15. Percent mortality of house flies exposed to MaxForce spot fly bait applied to wood blocks and oven-aged at 60°C for 1, 4, 5, and 10 days.

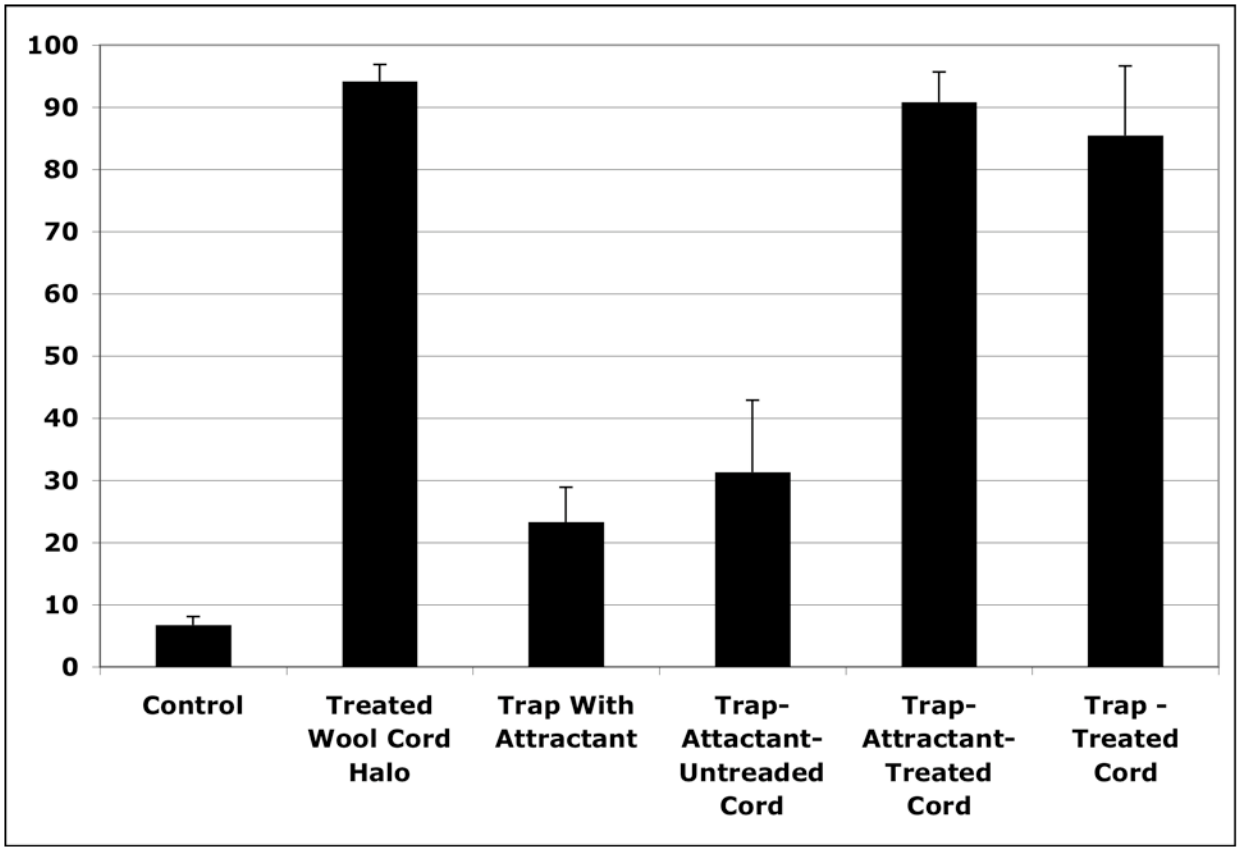

Figure 16. Percent mortality after 48 hours of house flies exposed in field screen cages to several combinations of MaxForce spot fly bait-treated wool cord and Trap N'Toss fly traps.

3. KEY RESEARCH ACCOMPLISHMENTS

Year 1 Research Accomplishments

- The following graduate students were hired and are researching aspects of flies and insecticides for deployed forces:
    - Matt Aubuchon, Ph.D. candidate
    - Lt. Ricky Vazquez (U.S. Army Reserve), Ph.D. candidate
    - Alexandra Chaskopoulou, M.S. candidate
    - Ryan Welch, M.S. candidate
    - HM1 Jeff Hertz (U.S. Navy), M.S.candidate
    - Kim Fererro, M.S. candidate
    - Enlisted assistance of Dr. David Williams as a research advisor

- A field strain of house flies was established in our laboratory and is being mass produced for testing.
    - Ammonia production by fly larvae is being investigated to improve rearing conditions.

- Facilities are now available for testing insecticides on flies.
    - Screened cages were purchased and set up for outdoor testing of insecticide formulations.
    - A wind tunnel was acquired for testing toxicity of contact insecticides.
    - University of Florida agreed to build a room to house the wind tunnel.

- Requests were disseminated for insecticides, insecticide formulations, and technologies among industry contacts.
    - Neonictotinoids, Pyrethriods, Phenylpyrazoles, Oxadiazines, and Organophosphates were submitted by manufacturers for evaluation.
    - Manufacturers who have submitted or are planning on submitting compounds for testing are FMC, Syngenta, DowAgroSciences, Wellmark/Zoecon, Dupont, BASF, Bayer, Sumitomo/MGK, and Whitmire.

- Primary bait toxicity testing was initiated on the neonicotinoid insecticides.
    - Because these are primarily water soluble, water/sugar based insecticide treatments determined oral toxicity using 3 assays: (a) Petri dish assay, (b) small cage assay, and (c) field cage assay. Initiated research on (a) traps and (b) attractants.

- Primary residual surface testing was initiated.
    - Dose-dependent mortalities of house flies to insecticide residues were determined.
    - Evaluation of pyrethroid/neonictotinoid combinations was conducted.

- Insecticide residues on fly cords were preliminarily evaluated with pyrethoid and phenylpyrazole insecticides.
    - A small field study with deployed forces in the Middle East.
    - Research on the preference of flies for color/gauge of yarn will be developed.

- Dose-dependent mortality of flies exposed to treated fly cord will be investigated.

Year 2 Research Accomplishments

- The following graduate student assistants studied flies and insecticides:
    - Matt Aubuchon, Ph.D., 2006.
    - Ryan Welch, M.S., 2006.
    - Lt. Ricky Vazquez (U.S. Army Reserves), Ph.D. candidate.
    - Alexandra Chaskopoulou, M.S. candidate.
    - HM1 Jeff Hertz (U.S. Navy), M.S.candidate.
    - Assistance of Dr. David Williams and Eugene Gerberg as a research advisors.
    - Assistance of Terry Kreuger and Osborne Willis as technicians.

- Field cages were established for testing insecticide outside performance.
    - Procedures for sampling flies and evaluating insecticide performance were established.

- Commercial cone trap testing procedure was developed and 6 commercial traps were tested.
    - The Trap n' TossTM (Farnam Companies, Inc., Phoenix, AZ) fly trap was the superior trap in performance.
    - Aged attractant mixture was more attractive than freshly mixed attractant.

- Light traps were tested to determine effect of time and incident light on fly catch.
    - Competing light sources significantly reduced catch.
    - Flies were caught within 1-2 hours of exposure to light traps.
    - Older flies are less attracted to light traps.

- Insecticide impregnated fly cords were evaluated.
    - Jute cord was a preferred resting cord, but wool cord provided greatest mortality.
    - Fipronil was found to provide reliable fly control on wool.

- Procedures were established and implemented to evaluate fly baits
    - Primary testing: Petri dish.
    - Secondary Testing: Small cage.
    - Field Testing: Large outdoor cages.
    - MaxForce Fly Spot Bait was evaluated in cooperation with Bayer Environmental, it is now EPA registered and should be available soon for military use.

- Dishwashing soap solutions were evaluated for fly control.
    - 2% soap sprays provided reliable and quick knockdown of flies.
    - 20% soap bait killed all flies after 2 hours.

- A wind tunnel was set up for testing toxicity of contact insecticides.
    - University of Florida built a room to house the wind tunnel.
    - Contact toxicity testing is being initiated.

- Testing was started on products submitted by industry. Compounds submitted were Neonicotinoids, Pyrethriods, Phenylpyrazoles, Oxadiazines, and Organophosphates. However, Ricky Vazquez, the student responsible for primary toxicity screening was deployed first to Egypt and now to Iraq.

Year 3 Research Accomplishments

- Evaluated vapor toxicity of volatile compounds to mosquitoes:
  - Formate esters were the most toxic family followed by the heterobicyclics and the acetate esters.
  - Methyl formate was the most toxic ester (LC50 = 1.36 mg/liter.
  - Coumaran was the most toxic heterobicyclic (LC50 = 2.03 mg/liter).
  - All compounds, except for menthofuran, were significantly more toxic to mosquitoes than Drosophila.
  - Formates showed highest toxicities to mosquitoes,, followed by heterobicyclics and acetates.
  - The best 7 performing compounds on mosquitoes were methyl, butyl, propyl, ethyl, and hexyl formate, and the heterobicyclics coumaran, and benzothiophene.

- Evaluated Vapor Toxicity of Volatile Compounds to House Flies:
  - The heterobicyclic menthofuran (LC50 = 3.70 mg/liter) was the most toxic of the new volatile compounds.
  - LC50 for EGDF was 9.27 mg/liter, and for heptyl formate 32.62 mg/liter of air.
  - Volatile compounds were approximately by 10X more toxic to house flies than Drosophila.
  - Controlled release of heptyl formate from ceramic rods caused high fly mortality for 9 days.

- Tested insecticide-impregnated cords for house fly control:
  - Manila cord attracted more flies than any other cord.
  - Synthetic cords attracted significantly less flies than the natural cords.
  - Animal-based cords were more attractive than the plant-based cords.
  - Highest fly mortalities were obtained with fipronil-impregnated or indoxacarb-impregnated wool cord.
  - The fipronil-impregnated cords were more lethal than the indoxacarb-impregnated cords.
  - Over 95% fly count reductions were obtained by 48 h with either fipronil- or indoxacarb-treated cords in field cages.

- Tested new imidacloprid bait for house fly control:
  - Imidacloprid granular and the imidacloprid sprayable baits caused higher fly mortality than the methomyl granular fly bait at 3 h.
  - At 24 h, the methomyl granular bait caused the highest fly mortality.
  - The imidacloprid bait-treated wool cord caused the highest fly mortality (74%) in the laboratory.
  - In field cages, the imidacloprid bait-treated cords caused >87% fly reductions by 24 h.
  - Fresh and 4-week aged had similar performance in reducing flies.

- National Stock Number Request for new imidacloprid fly bait:
    - Issue Paper was submitted to the Pesticide Committee of the AFPMB
    - Presentation to the Pesticide Committee by HM1 Joe Diclaro.
    - Recommendation approved by pesticide Committee and AFPMB Council.
    - New product available under NSN 01-555-9369.

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- Vazquez, R. J., P. G. Koehler & M. E. Scharf. (In preparation). Residual Toxicity of Neonicotinoids, Pyrethroids, and Pyrazoles to House Flies (Diptera: Muscidae). J. Econ. Entomol.

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Presentations

- Chaskopoulou A. & P. G. Koehler. Vapor toxicities of novel low molecular weight insecticides to mosquitoes and house flies. Entomological Society of America Annual Meeting. December 10^{th}-13^{th} 2006, Indianapolis, IN.

- Chaskopoulou A. & P. G. Koehler. New promising insecticides for mosquito and fly control. Deployed War-fighter Program (DWFP) Annual Review Meeting. March 6th-9th 2007, Gainesville, FL.
- Chaskopoulou A. & P. G. Koehler. Novel insecticides for mosquito control. The 4rth Arbovirus Surveillance & Mosquito Control Workshop. March 27th-29th 2007, St. Augustine, FL.
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- Chaskopoulou A. New larviciding methods for controlling mosquitoes. Master Gardener Graduation Ceremony. November 9th 2005, University of Florida, Microbiology Building, Gainesville, FL.
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- Welch, Ryan M. 2006 M.S. Entomology. Univ. of Florida.

5. CONCLUSIONS

During Year 1, we hired graduate students to conduct research on insecticides and flies. Facilities were established for rearing flies at the University of Florida and field strain of house flies has been established and mass-produced. Facilities for testing insecticides on flies were added to the lab and outside field screened cages were set up for testing. A wind tunnel was acquired for testing toxicity of contact insecticides, and is available for future work.

We contacted industry and notified them of our ability to test and evaluate new insecticides and technologies, and the need for new products for fly and mosquito control. Several insecticides were submitted by the industrial collaborators, including pyrethroids, neonicotinoids, phenylpyrazoles, oxadiazines, and organophosphates. Laboratory bait toxicity and attractant were initiated and followed by field cage evaluations. Preliminary residual surface testing was initiated, as was preliminary studies on the use of fly cords. A field test with deployed forces was conducted in Egypt.

One of our military students was deployed in Iraq during Year 2, which caused an interruption in the research doing for DWFP. Fly cone traps and fly light traps were evaluated to optimize military usage of these non-chemical controls. The research demonstrated that the Trap n' Toss™ (Farnam Companies, Inc., Phoenix, AZ) fly trap was better in attracting and catching flies and other traps tested. An aged fly attractant was better at attracting flies than the same attractant freshly prepared. Research on light traps documented that flies were caught within 1-2 hours of exposure to light traps, but competing light sources as those are commonly seen in food service areas for deployed troops, significantly reduced catch.

A sprayable spot fly bait was evaluated and the manufacturer submitted application for registration by EPA. All our tests indicated that this bait would be very useful for use by deployed troops. Further studies with this bait eventually led to the recommendation to the AFPMB Pesticide Committee and Council for an NSN assignment to this product (NSN 01-555-9369). We evaluated soap sprays as means to knock down fly populations safely. A 2% soap solution, which can be applied with a hand-held bottle sprayer directly to flies, provides immediate fly knockdown.

New volatile compounds, which are much safer for use around people than previously used products such as dichlorvos, were tested against both flies and mosquitoes. Heptyl formate was found to kill flies by vapor toxicity, but it volatilized rapidly giving no residual control. To overcome the rapid volatilization, controlled release through the use of porous ceramic rods was evaluated with this product. Results from this research demonstrated that these volatile compounds can be useful for control of mosquitoes and flies in confined areas when applied with adequate devices. Development of delivery devices needs to be considered in future research.

Because of changes in the research direction and promising results obtained with fly baits and volatile compounds, research that was planned for residual surface tests in treatment rooms Peet Grady Chambers was not performed. Instead, research was redirected to lab studies of volatile compounds, further studies on baited and unbaited traps, and testing of new fly baits and delivery methods for these products, such as the use of treated cords and the combinations of these materials with traps.

Testing of insecticide-impregnated cords for house fly control provide useful information on how to deliver insecticides to fly populations in a way that minimize exposure to pesticides of

troops and local populations in deployment areas. Wool cords were shown to be the best material for delivery of the test products, not because most flies get attracted to this material, but because acquisition of pesticide by the insects from wool cords seems to be more efficient. The presence of natural oils in wool cords seems to be responsible for the increased efficacy of wool cords in relation to other cords tested for delivery of fipronil, indoxacarb and imidacloprid. In the absence of wool cords, other natural cords may used as substitute. However, positive results with wool cords suggest the need for further research on the possible use of natural oils in the delivery of contact insecticides in traps and other delivery systems.

During the 3-year research project, research on control of mosquitoes and flies developed from the initial screening of insecticidal active ingredients to a field testing of new formulations and new application devices. The research addressed major tasks and milestones in the Statement of Work for the grant. To arrive at a product with a NSN and available for use by the military, the following steps were followed: In year 1 (2004-2005), primary toxicity testing was performed with several active ingredients. In year 2 (2005-2006), primary insecticide testing was refined and secondary testing focused on most promising formulations. Initial tests with application devices (lattices and cords) were preformed with new active ingredients not yet registered for fly control. The development work was concluded in year 3 (2006-2007) with small-scale field studies utilizing delivery devices that can be used where troops are deployed. Also, we performed stability tests of the formulation ultimately approved for an NSN by the AFPMB. Other research efforts complemented the main line followed to provide new products for control of mosquitoes and flies in deployment situations. Other active ingredients continue t be further developed and products are expected in the near future.

We have supported six graduate students to research insecticides and flies. Three of the students are in the military: Army Reserve Lt. Ricky Vazquez was deployed for 15 months in Iraq and is now back in our laboratory working on research for his dissertation. Navy LTJG Jeff Hertz has completed his degree and is now stationed at the Naval Entomology Center of Excellence in Jacksonville, FL. Navy HM1 Joe Diclaro is currently pursuing a M.S. degree in our laboratory working with fly control using insecticide-treated cords and traps.

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7. APPENDICES

Chaskopoulou thesis

Hertz thesis

TESTING VAPOR TOXICITY OF FORMATE, ACETATE, AND HETEROBICYCLIC COMPOUNDS TO *Aedes aegypti* AND *Musca domestica*

By

ALEXANDRA CHASKOPOULOU

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2007

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By

Alexandra Chaskopoulou

August 2007

Chair: Philip Koehler
Major: Entomology and Nematology

Volatile insecticides, commonly known as fumigants, have been widely used for the management of structural pests and for the protection of stored agricultural commodities. However, they have been mostly overlooked for the control of medically important pests such as mosquitoes and flies. Dichlorvos (DDVP) is the one volatile insecticide mostly studied on mosquitoes and flies. DDVP has been characterized by the Environmental Protection Agency of the United States as a "probable human carcinogen" and because of its implications in human health, in 2006 its use was restricted to confined spaces such as wardrobes and closets. Therefore, there is need to replace DDVP with friendlier and less toxic chemistries.

For my research I evaluated vapor toxicity of a series of new, promising, highly volatile chemicals with insecticidal activity, low mammalian toxicity, pleasant odors, and potentially novel modes of action on mosquitoes, using *Aedes aegypti* (L.), and on filth flies, using *Musca domestica* (L.). A total of 16 insecticidal compounds, 7 formates, 4 acetates, 4 heterobicyclics, and the organophosphate DDVP were tested on mosquitoes. DDVP was by far the most toxic compound, and specifically it was 54.4 times more toxic than the second best performing compound, the formate ester methyl formate. Within the novel compounds, overall, formate esters were the most toxic family, followed by the heterobicyclics, and last by the acetate esters.

The seven best performing novel compounds with vapor toxicity on mosquitoes were methyl formate>butyl formate>propyl formate=ethyl formate>hexyl formate>coumaran>benzothiophene. There were several structure-activity relationships observed. The most striking one involved the length of the aliphatic chain of the formate esters; as the length of the aliphatic chain increased, toxicity in general decreased. Also, the formate group within the aliphatic chain was correlated with higher toxicity than the acetate group.

A total of 4 compounds, the formate esters heptyl formate and ethylene glycol di-formate (EGDF), the heterobicyclic menthofuran, and the organophosphate DDVP were tested on house flies. DDVP was 25 times more toxic compared to the second best compound, the heterobicyclic menthofuran. Menthofuran was followed by EGDF, and last by heptyl formate. Also, ceramic porous rods were embedded with heptyl formate in order to evaluate the effectiveness of controlled vapor release of heptyl formate in killing house flies over time. It was shown that controlled vapor release of heptyl formate can be used successfully to provide house fly mortality over time.

Three of the novel compounds, heptyl formate, EGDF, and menthofuran were synergized with the insecticide synergists SSS-tributyl-phosphorotrithioate (DEF), and piperonyl butoxide (PBO), which are esterase and P450 inhibitors, respectively. For both mosquitoes and house flies, when EGDF and heptyl formate were co-applied with DEF their toxicities decreased, supporting esterase based activation of formate esters. Also, when menthofuran was synergized with PBO its toxicity increased, supporting P450 based deactivation of heterobicyclics.

CHAPTER 1
INTRODUCTION

It would be impossible to refer to all those times that insects affected the course of human history. Just to name a few, the death of great warriors like Alexander the Great was attributed to malaria, a disease transmitted by mosquitoes. Great empires like the Roman Empire were brought to decline because of the Bubonic plague, a disease transmitted by fleas. Of course not to forget that Bubonic plague, also referred to as the Black Death, was responsible for causing 25 to 75 million deaths in Europe alone. A vast number of deaths during wars is attributed to insect borne diseases; in the American civil war an estimate of 40,000 to 100,000 deaths were attributed to dysentery, a disease transmitted by filth-flies (Capinera 2004).

Because of the major impact insects can have on people's lives, people have had an ongoing battle with them since the very early years in the pages of history. The first human attempt to control insects was documented during the early years of ancient Greece. Homer described how Odysseus fumigated a house with burning sulfur to control insect pests (Homer, 800 B.C.E). Since then, there has been a lot of improvement in the development of chemical compounds with effective insecticidal activities. The first successful compound with phenomenal insecticidal activity was the chlorinated hydrocarbon, DDT (dichlorodiphenyltrichloroethane) (Casida & Quistad 1998). DDT was brought into the insecticide market in 1939, and it was effective against a wide range of insects and most notably against mosquitoes. Paul Muller received a Nobel Prize in 1949 for discovering the insecticidal activities of DDT (Capinera 2004). After the development of the chlorinated hydrocarbons other successful insecticidal groups followed, such as the organophosphates [parathion (1946), malathion (1952), chlorpyrifos (1965)], the methyl carbamates [carbaryl (1957), alanycarb (1984)], and the pyrethroids [allethrin (1949), resmethrin (1967), permethrin (1973), deltamethrin (1974)] (Casida & Quistad

1998). Some more recent insecticidal groups are the insect growth regulators such as juvenoids and chitin synthesis inhibitors [methoprene (1973), fenoxycarb (1981),] the chloronicotinyls [imidacloprid (1990)], the phenylpyrazoles [fipronil (1992)], and the avermectins [abamectin (1981)] (Casida & Quistad 1998).

All these insecticides have had a wide application spectrum targeting various insect pests, including agricultural and public health pests. My research will specifically focus on two of the most important categories of public health pests: the mosquitoes (Diptera: Culicidae) and the filth flies (Diptera: Muscidae). Each one of these pests has its own life history, unique behavioral and morphological traits, and different potential for disease transmission. Mosquitoes, despite their miniature size, and their delicate, vulnerable figure have managed successfully to survive on planet earth for more than 170 million years. With their unique adaptation mechanisms they have managed to thrive in almost all kinds of water habitats, from crab-holes and leaf-axils, to subzero tundra wetlands in Arctic. Mosquitoes are vectors of serious and deadly diseases such as malaria, yellow fever, dengue and the different types of encephalitis. A total number of approximately 320 million human cases of mosquito borne diseases with 2 million deaths occur every year (Tabachnick 2004). There are approximately 3,200 recognized mosquito species worldwide and the largest number of them still remains to be discovered (Rutledge 2004). The mosquito species that was studied in this research was the yellow fever mosquito *Aedes aegypti* (L). Like all dipterans, mosquitoes exhibit holometabolous development. Their life cycle is completed in two different environments: one aquatic and one terrestrial. The first three stages of their life cycle, the egg, the larva, and the pupa, are adapted to survive in aquatic environments, whereas the last stage, the winged adult inhabits terrestrial environments. Their life-cycle lasts from 7 to 14 days depending on the mosquito species. A more detailed description of the species'

morphology, behavior, biology and a review of the current control methods will be given in Chapter 2.

Filth flies have been in close association with humans since humans showed up on planet Earth. They have been a nuisance with their painful bites and a plague due to the serious, life threatening diseases they transmit. Some of the diseases they transmit are typhoid fever, dysentery and diarrhea. Flies are also known to infect human and animal flesh, a condition known as myiasis. There are approximately 87,000 species of flies worldwide (not including mosquitoes) (Scott & Littig 1964) and 9,000 of them belong to the family Muscidae (Mullen & Durden 2002). The filth fly species that was studied in this research was the common house fly *Musca domsestica* (L.). Houseflies have complete metamorphosis, and their life cycle is divided into 4 stages: the egg, the larva, the pupa, and the winged adult. The time necessary for the completion of the cycle depends on the species and on the environmental conditions such as temperature and moisture. A more detailed description of the species' morphology, behavior, biology, and a review of current control methods will be given in Chapter 3.

A major problem that emerged from the use of insecticides is the development of resistance. It was Melander (1914) that first reported insecticide resistance. Since then the number of insects and mites worldwide that have developed resistance to one or more pesticides has increased to 504 and continues to increase (Becker 2003). Specifically, the number of public health pests that developed insecticide resistance has increased from 2 in 1946, to 198 in 1990 (Oppenoorth 1985, Georghiou 1990). Both mosquitoes and houseflies developed resistance rapidly to various insecticides. Hemingway and Ranson (2000) gave a very nice review of insecticide resistance on mosquitoes that vector diseases. In 1947 the first case of DDT resistance was documented in *Aedes tritaeniorhynchus* and *Aedes solicitans*. Since then more

than 100 mosquito species have developed resistance to one or more of the insecticides discussed above. A broad-spectrum of organophosphate resistance or malathion-specific resistance has been documented in the major malaria vectors (*Anopheles* group) such as *Anopheles culicifacies, Anopheles stephensi, Anopheles albimanus, Anopheles arabiensis, Anopheles sacharovi.* Also, pyrethroid resistance has occurred in *Anopheles albimanus, Anopheles stephensi*, and *Anopheles gambiae* among others, not to neglect the carbamate resistance in *Anopheles sacharovi* and *Anopheles albimanus*. Widespread resistance to organophosphates has occurred in the *Culex* group as well, and pyrethroid resistance was recorded in *Culex quienquefasciatus*. Widespread resistance to pyrethroids has occurred in *Aedes aegypti*, and additionally many cases of carbamate and organophosphate resistance have been recorded as well. Things do not look any better for house flies. It was again in the year of 1947 that the first case of house fly resistance to DDT was recorded (Georghiou 1972). Keiding 1999 prepared a very nice review of the global status of insecticide resistance in field populations of the housefly, *Musca domestica* (L.). According to his review, when the organochlorines failed to control flies (1950), they were replaced by the organophosphorous compouds, and it wasn't long afterwards that organophosphorous resistance was recorded (1955, Denmark). It didn't take long to spread to different parts of the world (North America 1966, United Kingdom 1977, Germany 1979, Japan 1979, Belgium 1981, West Africa 1979, Australia 1989 to name a few). Widespread resistance to carbamates was also seen, with an early Czechoslovakian report in 1983. Resistance on pyrethroids was first recorded in Denmark in the 1970's. It is, also, worth mentioning that in the USA, the first pyrethroid resistance case was observed in 1984 in Georgia after only 2 years of permethrin use.

Considering the limited number of insecticides registered for management of public health insect pests and the increasing incidents of resistance documented, it is essential that already existing insecticides must be used wisely and that new insecticides with novel modes of action must be discovered. The main objective of this study is to evaluate a series of new promising chemicals with insecticidal activity and potentially novel modes of action on mosquitoes, using *Aedes aegypti* (L.), and on filth flies, using *Musca domestica* (L.). These new insecticides have high vapor pressures, and, as a result they show potential to act as vapor toxicants. The experiments presented in this paper evaluated vapor toxicity of the novel insecticidal chemistries.

CHAPTER 2
LITERATURE REVIEW: THE YELLOW FEVER MOSQUITO

> No animal on earth has touched so directly and profoundly the lives of so many human beings. For all of history, and all over the globe, she has been a nuisance, a pain, and an angel of death. The mosquito has killed great leaders, decimated armies, and decided the fate of nations. All this, and she is roughly the size and weight of a grape seed. (Spielman and D'Antonio 2001, p. 15 from The Preface to Mosquito)

Classification and Distribution

Aedes aegytpi (L.) is a mosquito species in the family Culicidae, subfamily Culicinae, and tribe Aedini. There are three types of this species: the typical form *Ae. aegypti aegytpi*, *Ae. aegytpi queenslandensis*, and the smallest type *Ae. aegytpi formosus* which is a forest species (Nelson 1986). Only the first two types are found in the USA.

World distribution. *Ae. aegypti* is thought to have originated from Africa (Gratz 1993). It has been introduced to many parts of the world through ships and therefore ports are the first areas to be invaded. Currently this species is distributed in most tropical and subtropical world regions, with a range extending from 40 degrees N to 40 degrees S latitude (Womack 1993).

USA distribution. *Ae. aegypti* occurs in 21 states, which are Alabama, Mississippi, Florida, Georgia, Tennessee, Kentucky, North Carolina, South Carolina, Virginia, New York, Delaware, Maryland, Kansas, District of Columbia, Illinois, Arkansas, Louisiana, Missouri, Oklahoma, Texas, and New Mexico (Womack 1993, Darsie and Ward 2005).

Florida distribution. *Ae. aegypti* used to be widely distributed through the entire state of Florida (Tinker and Hayes 1959, Morlan and Tinker 1965). However, since the introduction of *Ae. albopictus* in 1986 (Peacock et al. 1988), a significant decline of the *Ae. aegytpi* population has been detected (O'Meara et al. 1992a, O'Meara et al. 1995).

Morphology

The Egg

The eggs are black in color, cigar shaped and one millimeter in length.

The Larva

The body of the larva is divided into 3 distinct segments; the head, the thorax and the abdomen. The head and thorax have an ovoid shape and the abdomen is divided into nine segments. The posterior segment of the abdomen has four specially modified gills for osmotic regulation and a siphon specialized for breathing (Mullen and Durden 2002). The siphon of the *Aedes* mosquitoes is distinctively shorter than other mosquitoes and plays an important role in distinguishing them from others. Also, the position of the *Aedes* larvae in water is almost vertical to the water surface (Nelson 1986).

Two distinctive characteristics set *Ae. aegytpi* apart from other *Aedes* larvae. The first one is the two prominent lateral hooks (spines) on each side of the thorax. The second one is the row of seven to twelve comb scales on the eighth abdominal segment. Each one of these scales has two lateral teeth and a medial spine that gives it a 'pitchfork' appearance (Nelson 1986, Darsie and Ward 2005).

The Pupa

Pupae in the genus of *Aedes* have a distinct short hair at the tip of each swimming paddle and short breathing tubes known as air trumpets (Nelson 1986).

The Adult

Aedes aegypti is a medium sized black colored mosquito with a distinctive lyre-shaped design on the mesonotum. It also has white bands at the bases of the tarsal segments. Another key characteristic is the white segments on the palpi and the clypeus (Christophers 1960, Darsie and Ward 2005).

Life Cycle

The Egg

Aedes aegypti were originally tree-hole breeders (Soper 1967), but as they evolved and adapted in environments near and around human dwellings they became container breeders. A unique characteristic of their biology is that they attach their eggs on the sides of artificial as well as natural containers (Pratt and Littig 1967, Nelson 1986, Mullen and Darden 2002, Becker 2003, Rutledge and Evans. 2004). The eggs are fertilized at the moment of oviposition and it takes from 48 hours up to 5 days for embryonic development to be completed depending on the environmental temperature (Nelson 1986). The eggs have the ability to withstand long desiccation periods for up to one year and sometimes even more. Temperature and humidity play a significant role in the viability of the eggs. It was shown that at relative humidities from 91% to 95% *Ae. aegypti* embryos can survive for up to 15th months (Christophers 1960). Also, temperatures ranging from 42^{o} to 53^{o} F were shown to be lethal to the embryo when the eggs were exposed to them for more than 2 weeks. Flooding is the necessary stimulus for the eggs to hatch. It takes 15 minutes of flooding for some eggs to hatch. On the other hand some eggs need to be inundated several times prior to hatching (Nelson 1986).

The Larva

The larval development is divided into 4 instars. The first three instars develop fast and are more sensitive whereas the last instar takes longer to develop and increases more in size and weight (Nelson 1986, Mullen and Durden 2002). The duration of the development depends on several factors such as food availability, environmental temperature and larval density (Cristophers 1960, Gerberg et al. 1994). It can vary from as short as 5 days at optimal conditions up to 14 days. At a constant temperature of 21-25^{o} C the larvae are expected to pupate at 10-12 days (Geberg et al. 1994). Under unfavorable conditions the duration of the last instar can last for

up to several weeks before pupation takes place (Nelson 1986). The male larvae develop faster than the female larvae, and as a result they pupate one day earlier (Mullen and Durden 2002).

The Pupa

The pupae are the least active stage. They do not feed and their main function is metamorphosis into the mature adult stage (Nelson 1986, Mullen and Durden 2002). The pupae have the ability to react to external stimuli such as vibrations and light, and thus actively move away. The pupae are buoyant, and therefore they have the ability to float on the water. This property allows them to emerge as adults. The duration of the pupal stage lasts 2 to 3 days (Nelson 1986, Mullen and Durden 2002). The male pupae develop faster than the female pupae.

The Adult

Emergence

At the early stages of brood emergence males are most abundant (Christophers 1960). When emergence is completed, the adult rests at the sides of the container for a few hours until the wings and the exoskeleton harden and darken (Nelson 1986). Additionally, the males have to rotate their genitalia 180^{o} into the right position (Nelson 1986, Becker 2003). This may last up to 24 hours.

Mating

Approximately 24 hours after emergence, mating takes place. Mating takes place with the female at rest or in flight (Schoof 1967). The males are attracted to the females due to the sound that is made by their wing beat. Females can begin to produce the desirable wing beat 2.5 hours after emergence (Roth 1948, Nelson 1968). The attracted male clasps the tip of the female abdomen with his genitalia and inserts his aedeagus into the female genital chamber. The duration of the copulation is brief and lasts less than a minute (Roth 1948). Females mate once since one insemination is enough to fertilize all the eggs that a female will develop in her

lifetime. Males on the other hand were shown to mate up to 10 and 15 times (Schoof 1967). After mating is complete the female searches for a blood meal. Once blood fed the female no longer emanates the wing beat tone (Roth 1948, Nelson 1968).

Feeding

The male mouthparts are not adapted for blood feeding. They meet their energy requirements by feeding on flower nectar. Females also feed on flower nectar to satisfy their carbohydrate needs. The female requires additionally a protein rich blood meal in order to be able to develop viable eggs (Magnarelli 1979, Clements 1992). The females of *Aedes aegypti* show preference in feeding on humans, a behavior known as "anthropophilic" (Carpenter and LaCasse 1955); however, they will feed on most vertebrates when available. Female mosquitoes use several stimuli to detect and reach their host. Carbon dioxide, octenol and lactic acid are some of the most documented host attractants (Acree et. al 1968, Takken and Kline 1989, Mboera and Takken 1997). Female mosquitoes fly upwind following the odors and other attractants released by the host. Once they are in close proximity to the host they use visual cues to locate the host. It was shown that *Ae. aegypti* are more attracted to black surfaces (Brett 1938) and to black-white interfaced surfaces (Brown 1966). As they approach even closer, temperature and other skin emissions guide them to the proper feeding site. Blood feeding usually takes place during daylight (Nelson 1986).

Flight range

Males fly less than the females (Nelson 1986). A female *Aedes aegypti* more commonly remains at the location where it emerged. In an experiment done by Trips and Hausermann (1986) it was shown that most marked *Ae. aegypti* were caught in the house in which they were released. When needed, a female can fly up to 2.5 kilometers in search of breeding sites

(Wolfinsohn and Galun 1953). It has been estimated than on average one female does not exceed 50 meters of flying during its life time (Nelson 1986).

Resting behavior

The most suitable resting place is a dark, quiet place. They mostly prefer to rest beneath and inside structures and rarely choose to rest outdoors on vegetation (Schoof 1967, Nelson 1986). They generally show preference resting on vertical surfaces.

Longevity

Aedes aegytpi adults in a laboratory setting can live for several months varying from 131 up to 225 days (Christophers 1960). However, in nature they usually survive for only a few weeks. Previous work has shown an average life-spam of 15 d for female mosquitoes outdoors (Nelson 1986). It is estimated that, when a population emerges, 50% of the adults die on average during the first week and 95% of the population dies after the first month. However, if the beginning emerging population is large, the subsequent older population will be adequately large to transmit disease and initiate an epidemic (Nelson 1986).

Fecundity

After a complete blood meal (2-3 mg), a female will produce and oviposit ~100 eggs (Nelson 1986). Smaller meals result in the production of small batches of eggs. It takes three days between blood engorgement and egg oviposition. It is also worth mentioning that a female can feed again the same day that oviposition took place. A single female can produce several egg batches in its life-time.

Public Health Importance of the Yellow Fever Mosquito

Aedes aegytpi is the main vector of 2 serious and life-threatening diseases, yellow fever, and the two forms of dengue, dengue fever (DF) and dengue hemorrhagic fever (DHF). Both diseases are caused by viruses in the family Flaviridae (Mullen and Durden 2002).

Yellow fever. It is caused by the YF virus. The relationship between Ae. aegytpi and YF virus was confirmed through the work of Carlos Finley (1881) and Walter Reed (1900). This discovery was of great importance, and it was the initiation of serious mosquito control measures to eradicate the mosquito vector, which brought great results and decrease significantly the vector populations. Currently, yellow fever is a serious threat in Central America, South America and lowland equatorial Africa. Yellow fever is the cause of approximately 30,000 deaths every year (Tabachnick 2004). The latest epidemic in the United States was in 1905 in New Orleans, where there were 3,402 cases and 452 deaths (Mullen and Durden 2002). Yellow fever is a hemorrhagic disease. Symptoms start to appear 3-6 days after infection. There are several cases of yellow fever with mild or no symptoms at all (Shroyer 2004).

Dengue fever (DF) and dengue hemorrhagic fever (DHF). Dengue is caused by the DEN virus that exists in 4 different and distinct serotypes (DEN-1, DEN-2, DEN-3, DEN-4). There are two forms of disease, the classic dengue fever and the most severe form the dengue hemorrhagic fever. Some of the symptoms of dengue are fever, headache, rash, and pain in the muscles and joints (Mullen and Durden 2002). The symptoms of the disease can vary from mild to fatal. The severity of the symptoms depends on the age as well as the infection history of the patient. Children show higher fatalities (CDC 2005). The first epidemic of DF that was reported occurred in 1779-1780 in three different continents simultaneously: Asia, Africa, and North America (CDC 2005). Dengue is responsible for hundreds of thousands of cases every year (CDC 2005). Specifically, from 1956 to 1980 there were 715,238 cases of DF and 21,345 deaths reported, and from 1986 to 1990 there were 1,263,321 cases and 15,940 deaths (Rigau-Perez et al. 1994).Currently, this disease is a problem to all tropical and subtropical areas of the world.

Indigenous transmission of the disease in the USA was reported in the years of 1980, 1986, and 1995 in Texas (Rigau-Perez et al. 1994, Mullen and Durden 2002).

Control Methods of the Yellow Fever Mosquito

Every organized mosquito control program is composed of 3 main components: surveillance of the mosquito target, methods for controlling the immature mosquito stages and methods for controlling the adult mosquitoes.

Surveillance

Surveillance is the basis of every pest control program. Constant knowledge of the distribution and composition of mosquito populations is the key to a well organized and effective control program. Also, pre- and post-treatment surveillance is necessary in order to evaluate the success of every control method implemented. There are various tools and methods available to monitor mosquito populations. When still in the immature stages the most common monitoring method is the dipping technique using a standard dipper, a dipper with a screened bottom or a cooking buster (Schreiber 2004). There are some monitoring techniques modified specifically for monitoring *Ae. aegypti* larvae. Harrison et al. (1982) and Undeen & Becnel (1994) developed 2 different types of floating traps specialized for collecting *Ae. aegytpi* larvae. When in the adult stage the surveillance of the mosquito populations is accomplished in two main ways: through the human landing rate technique and through the use of trapping devices. The most commonly used trap is the dry ice baited CDC trap with or without ice (Schreiber 2004). The New Jersey light trap is also used; however, when used in urban settings where *Ae. aegypti* are predominantly found, the lights from the houses will compete with the trap light source resulting in smaller numbers of mosquitoes captured (Schreiber 2004). Fay (1968) designed a trap, called the Fay trap, for specifically collecting *Ae. aegypti* adults. The Fay trap is similar to the CDC trap except that it is painted shiny black with the light source replaced by a glossy black board.

Methods for Controlling Immature Mosquitoes

The different methods available for controlling the immature mosquito stages are applied directly in the water and they can have larviciding action, pupiciding action, or they can even kill the adult mosquito while it is emerging. Some common methods for controlling immature mosquitoes are source reduction, use of the mosquito fish, *Gambusia affinis,* which is a form of biological control, use of bacterial insecticides such as *Bacillus thuringiensis israelensis* (B.t.i.) and *Bacillus sphaericus* (B.sph.), use of insect growth regulators (IGR's) such as chitin synthesis inhibitors and juvenile hormone analogues, use of surface control agents such as oils and monomolecular films, and use of insecticides such as the organophosphate insecticide temephos. Source reduction is one of the most effective methods for controlling container breeding mosquitoes such as *Ae. aegytpi*. Gubler et al. (1991) pointed out that "the only truly effective way to control mosquito vectors of dengue is source reduction". The mosquito fish was used in Malaysia in water containers for the control of *Ae. aegypti* (Becker et al. 2003). B.t.i. and B.sph. are two different species of naturally occurring soil bacteria capable of producing, during their sporulation, proteins that are toxic to mosquito larvae. The larvae need to be actively feeding on the bacterial spores in order for the product to be effective. B.t.i and B.sph. are available in different formulations such as liquids, powders, granules, tablets and briquets. B.t.i. is more effective in controlling *Aedes* and *Psorophora* species (Weinzierl et al 2005). B.sph. is effective in controlling *Culex*, *Psorophora* and *Culiseta* species (Weinzierl et al 2005). Its effectiveness in controlling *Aedes* species varies, for example it is not as effective in controlling *Ae. aegypti* populations. It was shown that *Ae. aegypti* larvae were 100 times less susceptible to B.sph. compared to other mosquito species (Becker 2003). A distinct difference between B.t.i. and B.sph. is environmental persistence. B.sph. can persist in the environment whereas B.t.i. has little residual activity. A new tablet formulation of B.t.i. and B.sph. was successfully used to control

Cx. p. pipiens and *Ae. aegypti* (Becker et al. 1991, Kroeger et al. 1995). Timing of application for both bacterial species is very critical, because early first and late fourth instar larvae do not feed and thus they will not receive the chemical. Diflubenzuron, a chitin synthesis inhibitor, interferes with the molting process of the larva and prevents the normal development of the cuticle (Becker et al. 2003). Methoprene is an analog of a naturally occurring insect hormone called juvenile hormone. Methoprene works by interfering with the mosquito's life-cycle. By doing so it prevents the insect's metamorphosis from an immature to an adult and causes adult sterility. Methoprene gets absorbed on contact through the larval integument, thus larvae don't need to be feeding in order for methoprene to act effectively. Methoprene is commercially available with the name Altosid. Altosid products come in different formulations such as liquids, powders, granules and briquets. Altosid formulations are known for their long residual activity for up to 150 days (Florida Coordinating Council on Mosquito Control 1998). Some commonly used surface agents are the Golden Bear oil and the monomolecular films Arosurf MSF and Agnique MMF. Surface oils cause mortality to mosquito larvae and pupae through suffocation because the oily surface prevents the insects from obtaining air through their siphon. On the other hand the monomolecular films prevent the insects from remaining on the surface of the water by reducing the tension of the water surface. Under these conditions larvae and pupae die from exhaustion as they use up their energy reserves trying to stay at the surface. Temephos is a heterocyclic organophosphate and is widely known with the commercial name Abate. It is available in different formulations such as liquids and granules. Temephos is very effective against all mosquito species and has a very low mammalian toxicity with an LD50 of 2030 mg/kg. It acts by inhibiting the activity of acetylcholinesterase enzyme in the Central Nervous System (CNS) synapses resulting in the accumulation of acetylcholine at its post-synaptic receptor. The excess

of acetylcholine causes neuroexcitation, rapid twitching of the muscles, and final paralysis of the insect. Temephos has been used successfully to control *Ae. aegytpi*. For example, in Thailand an up to 95.4% reduction of adult density was achieved after applying temephos 1% granule formulation on the bodies of water containing larvae (Gratz 1993, Becker et al. 2003). However, resistance to temephos has been reported (Grandes and Sagrado 1988) and is a serious concern.

Methods for Controlling Adult Mosquitoes

Chemical control of adult mosquitoes, commonly known as "adulticiding", is divided in two main categories based on behavioral traits of the mosquito: Control of the resting adults, which are residual applications referred to as barrier or surface sprays, and control of the flying adults, which are Ultra Low Volume applications referred to as space sprays. These two categories differ in the type of insecticides that are utilized as well in the application techniques that are used to distribute the insecticides on the target insect. Additionally, there is also one less popular approach available for controlling adult mosquitoes, which involves the use of vapor toxicants.

For the control of the resting adults, also, known as barrier treatment applications, residual insecticides are applied to perimeters around private residencies and recreational areas where mosquitoes are anticipated to rest. Some of the commonly used insecticides are deltamethrin, bifenthrin, betacyfluthrin, and lambda-cyhalothrin. Barrier treatments are large droplet applications, commonly applied during daylight hours, and are anticipated to last from a week up to two months depending on the insecticide used. Reiter (1991) pointed out that resting behavior of *Ae.aegytpi* plays a key role on the control of the insect, because unlikely most mosquito species, *Ae. aegytpi* prefer to rest inside (endophilic behavior) or around houses, and therefore they are hard to target through space-spraying applications. In agreement to Reiter's theory,

Chadee (1990) found that residual house spraying, a surface spray, was more effective for controlling *Ae. aegytpi* compared to ULV (ultra low volume applications) space spray.

The control of the flying adults is the most visible type of treatment with immediate results, and is the method of choice when there is a need for rapid reduction of mosquito populations like in the case of a disease outbreak. For this type of treatment the target is the flying adult mosquito and therefore the timing of spraying must coincide with mosquito flight activity. The treatments can be applied either aerially or by ground. The application technique is called Ultra Low Volume (ULV). ULV technology; as defined by the Environmental Protection Agency, is a method of dispensing insecticide in volumes less than 5 liters per hectare. Within mosquito control concentrate insecticide is often applied, therefore the output volume can be even lower < 1 liter per hectar. In other words ULV is a technique that applies the minimum amount of liquid of insecticides per unit area. The size of the droplets within the insecticidal cloud plays a very important role in determining the effectiveness of every spraying mission. Previous research has shown that the optimum droplet size for adult mosquito control is 5-10 microns (volume median diameter) for ground applications and 10-25 microns (volume median diameter) for aerial applications (Mount 1970). The size of the droplet determines the number of droplets per unit volume of insecticide, the time of which a droplet remains airborne, and the chances of the droplet penetrating through obstacles such as vegetation to reach the mosquito target (Becker 2003). Some common insecticides that have been used for controlling adult mosquitoes are fenthion, malathion and naled of the organophosphate family, sumithrin and resmethrin of the first generation synthetic pyrethroids and permethrin of the second generation synthetic pyrethroids (Florida Coordinating Council on Mosquito Control 1998). There has been a certain degree of failure of space spraying applications in controlling *Ae. aegypti* adults (Fox

1980, Perich et al. 1990, Gratz 1993) and a suggested explanation to that is their tendency to rest indoors (Becker 2003).

One last approach for adult mosquito control involves the use of slow release vapor toxicants. This is one of the least popular control methods and there has only been little research conducted to test the effectiveness of such applications. This could likely be attributed to the lack of insecticidal compounds with effective vapor toxicities. Dichlorvos (DDVP) is the one insecticide that has been most studied as a vapor toxicant against mosquitoes and other medically important pests (Maddock et al. 1963, Brooks & Schoof 1964, Brooks et al. 1965). Dichlorvos is an organophosphate insecticide and for the first time it was registered to be used as an insecticide in 1948 (EPA Pesticide Fact Sheet, 1978). A very common slow vapor release formulation of dichlorvos is resin strips. Slow release formulations of dichlorvos were shown to work effectively as an additional mosquito control method in occupied houses for malaria eradication programs (Mathis et al. 1959, Quarterman et al. 1963). However, the high acute mammalian toxicity of dichlorvos, in combination to reported resistance incidents has limited the use of dichlorvos as a widespread mosquito control method. Therefore, there is a need for new insecticidal compounds, with good vapor toxicities and novel modes of action that will replace dichlorvos. This research evaluated vapor toxicity of novel, low molecular weight, highly volatile formate, acetate, and heterobicyclic compounds on mosquitoes.

CHAPTER 3
LITERATURE REVIEW: THE HOUSE FLY

> And there came a grievous swarm of flies into the house of Pharaoh, and into his servant's houses, and into all the land of Egypt, and the land was corrupted by this kind of flies. (The Bible, Exodus 8:24, p. 74)

Classification and Distribution

The house fly *Musca domestica* (L.) belongs to the class Insecta, the order Diptera, suborder Cyclorrapha, and family Muscidae. It is commonly named house fly due to its close association to human settlements and activities. It is the most common fly in and around the home and it is a nuisance in every place where domestic animals are kept and waste accumulates. It is distributed around the world (West 1951) with the only exception of the Arctic, the Antarctic and areas of extreme high altitudes (Scott & Littig 1964). There are four different subspecies*: M. d. domestica* Linnaeus, *M. d. vicina* Macqvart, *M. d. nebulo* Fabricius, and *M. d. curviforceps* Sacca & Rivosecchi. The first three subspecies are found in temperate zones all over the world including subarctic and subtropical areas where as the fourth subspecies is limited to Africa (Keiding 1986).

Morphology

The Egg

They are 1-1.2 mm in length, banana shaped and creamy in color (West 1951, Keiding 1986).

The Larva

The larval stage is divided in three instars, from which the third one or else known as prepupa can reach up to 13 mm in length (Keiding 1986). Each instar is characterized by a cylindrical body divided in 13 well-defined segments with no appendages (West 1951). The larval head has no eyes and is located on the anterior, conical-shaped end of the larval body. For

feeding and for locomotion the larva has one strong and one small interior mouth hook located at the head. The posterior end of its body is rounded and consists of a pair of sclerotized structures, the spiracles, which are essential for breathing.

The Pupa

When the fly is ready for pupation, the integument of the third larval instar contracts and hardens to form a barrel shaped puparium (West 1951, Keiding 1986). The size of an average puparium is 6.3 mm in length (West 1951). For the first couple of hours the puparium is soft with a whitish, creamy coloration. As the cuticle hardens the color gradually darkens into a dark brown coloration.

The Adult

An adult house fly is approximately 6-9 mm in length and has a grayish coloration (West 1951, Mullen & Durden 2002). It has a pair of wings longer than the abdomen and when in rest they are directed posteriorly giving a triangular appearance to the fly (West 1951). The house fly's body is divided into three well defined regions: the head, the thorax, and the abdomen. The head has a pair of prominent eyes, where in the case of males are joined together (holoptic), and in the case of females are divided (dichoptic) (Mullen & Durden 2002). Adults have a pair of sucking mouthparts called the proboscis, which is composed of the labium that encloses the labrum and the hypopharynx and terminates in a two lobed labella (West 1951). The thorax is usually characterized by 4 dark, longitudinal stripes called vitae (Mullen & Durden 2002).

Life Cycle

The Egg

The eggs are laid in clusters in moist substrates of decaying, fermenting or putrefying organic matter (Schoof et al. 1954). One house fly can lay approximately 100-150 eggs (West 1951). The most favorable breeding sites are human waste and animal manure (Keiding 1986).

The eggs are very dependent upon moisture. It was shown that below 90% RH egg mortality increases (Keiding1986). Also, temperature plays an important role in the egg development. At 35^0C it takes 6-8 hours from oviposition to hatching. Below 13^0C and above 42^0C the eggs die before hatching (West 1951).

The Larva

The larval stage is divided into three instars. The first, second, and part of the third instar are called the feeding stages. They mainly feed on bacteria and their decomposition products. Odors attract the feeding stages to the breeding media. The larval stages tend to avoid light and prefer to occur in humid environments with a temperature around 35^0C (Keiding 1986). The late third instar is called prepupa and does not feed. In this stage the prepupae migrate to cooler and less humid environments where pupation takes place. There are several factors that affect the duration of the larval development such as nutrition, moisture, and temperature. Under optimal conditions it takes a minimum of 3-4 days for the completion of the larval development (Keiding 1986, Hogsette 1995).

The Pupa

The duration of this stage depends on humidity and temperature and lasts minimum of 3-4 days under optimal conditions (35-40C, 90% RH). The pupae have the ability to withstand lower humidity than the larvae. It has been shown that below 75% some pupae die and below 40% few survive (Keiding 1986).

The Adult

Emergence

When the development of the adult is completed within the pupal case, the adult brakes through the puparium and emerges quickly. The newly emerged adults are light grey and soft in appearance. Also, they have no wings. Before the newly emerged adults become fully capable of

flying they go through a phase that lasts several hours during which the cuticle hardens and the wings unfold (Keiding 1986). The young adults are ready for feeding 2-24 hours after emergence.

Mating

Males and females are ready for mating approximately 24 and 30 h, respectively, after emergence at optimal environmental conditions (Keiding 1986). Visual and olfactory stimulants are involved in the attraction between male and female adult flies (Colwell & Shorey 1977, Keiding 1986). A sex pheromone, (Z)-9- tricosene (muscalure), is produced by the females to attract the males (Carlson et al. 1971, Carlson & Leibold 1981). Also, another pheromone produced by the males is known to attract virgin females (Schlein & Galun 1984). Last, the wing beat frequency of the males was shown to have an effect on the mating behavior of the females (Colwell & Shorey 1976). Females usually mate once during their lifetime (monogamous) and store the sperm into the spermatheca (Keiding 1986), as opposed to males that can mate multiple times (polygamous).

Oviposition

Oviposition is closely dependent on air temperature. Below 15^0C no oviposition occurs (Keiding 1986, West 1951). Ammonia, carbon dioxide and other odors of rotting and fermenting materials attract the gravid females to their breeding medium (West 1956, Keiding 1986). Favorable breeding media include dung (Haines 1955), garbage and waste from food processing facilities (Schoof et al. 1954), sewage and accumulation of plant material (Silverly & Schoof 1955). The eggs are very sensitive to moisture and in order to be protected from desiccation are laid beneath the surface, within cracks and crevices. On average a female oviposits 120 eggs per batch (West 1951).

Feeding

House flies are considered to be polyphagous species, which means that they can feed on a wide variety of food material and they do not depend on particular types of proteins like other members of the family Muscidae (West 1951). Both male and female houseflies need water and sugars in order to survive. It is only the female flies that need additional protein in order to be able to develop viable eggs. They acquire their nutrients mostly from animal dung, human food and garbage. They are attracted to the food source mostly by visual cues. Odorous stimulants play some role when the food source is in close proximity (Keiding 1965). Flies are attracted to smells of fermenting and decomposing materials. When in contact with the food the fly uses special receptors on the legs and antennae to taste the food.

Longevity

Under laboratory conditions adult house flies can live up to a month (Keiding 1986). However, in field conditions the life span is considered to be less, approximately 2 weeks under ordinary conditions (West 1951).

Fecundity

A single female house fly produces approximately 120 eggs per cycle. The number of generations per year varies depending on the environmental conditions. At temperate climates house flies can produce up to 30 generations per year whereas in tropical climates the number of generations decreases to 10 per year (Keiding 1986). Theoretically, if a female fly laid 120 eggs in the middle of April, she would be responsible for the emergence of 5,598,720,000,000 flies in the middle of July (West 1951)!

Flight-range

House flies are strong fliers, can move forward at a rate of 6-8 km per hour, and don't tend to migrate (Keiding 1986). Provided that food and breeding medium is available they will remain

within a radius of 100-500 m from their breeding site. However, they have been shown to migrate up to 5-20 km from their breeding site (Schoof 1959, Keiding 1986, Nazni et al. 2005).

Public Health Importance of the House Fly

House flies, because of their behavior and biology can act as very effective disease vectors. They prefer to spend most of their life time on animal manure, human excrements, garbage and any type of decaying organic matter. However, they will eagerly utilize any other food source on any type of human facility that is available to them, and when that happens they will transfer pathogens from one substrate to the other. Houseflies are capable of transmitting pathogenic microorganisms through different modes of transmission. They can mechanically transfer them on the hair of their body (West 1951, Graczyk et al. 2005). They regurgitate them in their vomit, and they can also transfer them in feces through their alimentary track (Sulaiman et al. 2000). The pathogens transferred on the surface of the fly do not multiply, and they can only survive for a few hours. On the other hand, the pathogens in the alimentary track can multiply and survive longer for up to several days (West 1951). Therefore this mode of transmission is the most important and dangerous one.

The diseases that house flies transmit are intestinal diseases, eye diseases, and skin and wound diseases. Some examples of intestinal diseases are bacterial infections (shingellosis, salmonelosis, cholera), protozoan infections, and viral infections (poliomyelitis) (Levine & Levine 1991, Healing 1995, Mian et al. 2002, Graczyk et al. 2005). Outbreaks of diarrheal diseases in predominantly developing countries have been associated with the seasonal increase in abundance of filth flies (Graczyk et al. 2001). For example, in Thailand the seasonal peak in fly populations coincides with outbreaks of cholera (Echeverria et al. 1983). Examples of eye diseases that can be transmitted by houseflies are trachoma and conjunctivitis (Forsey & Darougar 1981). Last, an example of a skin disease is habronemiasis, a horse disease (Foil &

Foil 1988). This disease involves the deposition of infective house fly larvae onto mucous membranes of preexisting skin lesions on the stomach of horses.

Control Methods of the House Fly

Surveillance Methods

For every successful pest control approach it is vital to obtain information on the density and species composition of the pest population prior to any treatment. Post-treatment surveillance is necessary as well in order to evaluate the success of the control measures that have been implemented. There are several devices available for housefly surveillance that are commonly known as fly-traps. Fly-traps utilize visual stimuli and/or chemical attractants to lure flies. These could be ultra violet (UV) light traps which act as electrocutors, sugar/pheromone (sex pheromone-muscalure) baited traps, as well as cards or strips coated with sticky material to capture flies. Traps will not measure the absolute number of flies in a population, rather they will give an index and, also, the effectiveness of these traps to capture flies depends on their location within a certain area, temperature, and the physiological condition of the flies (Keiding 1986). Traps besides being a monitoring tool are also used for control operations.

Control Methods

Sanitation

A very old English quote says “Kill a fly in July, you’ve just killed one fly. Kill a fly in June, they’ll be scarced soon. Kill a fly in May, you’ve kept thousands away” (retrieved from West 1951). Within these 2 lines lies the very essence of a successful fly control plan. Due to their high reproduction rates, housefly populations can increase rapidly within a small period of time. Preventing the population from building up would be the best approach for effective and long-term fly control. The way to achieve prevention is to eliminate the conditions that allow flies to breed and multiply. Some examples of ideal housefly breeding media, as has been

discussed above, are animal manure, human feces, and garbage. Proper disposal of animal manure, human feces, and garbage is the primary and most effective method to control houseflies. Pickens et al. (1967) recommended frequent, if not daily, removal of animal manure. Barnard (2003) suggests collecting and storing manure in cone-shape piles to reduce the available surface area to flies. He also suggests proper composting or covering the organic matter with plastic to minimize fly attractiveness. West (1951) suggests storage of manure, when frequent disposal is not feasible, within concrete pits that will be fly-tight. Regarding disposal of human feces a properly operating sewage processing plant is necessary for each city and town (West 1951). Last, regarding garbage handling and disposal, open dumps must be replaced with sanitary landfills. In these landfills garbage will be compacted daily and covered with 24 inches of soil to effectively eliminate fly breeding (Keiding 1986). Another approach for treating garbage in large cities is complete combustion at temperatures of 1,400 ^{0}F to 2,000 ^{0}F, which would completely destroy organic material and prevent flies form breeding (Scott & Littig 1964). In conclusion, environmental sanitation is the best, long-term solution to every housefly problem.

Chemical control

For those situations where the fly population has already increased dramatically and immediate control is required there are several chemical based approaches that one could follow, which involve the usage of insecticides. There are, mainly, six different types of insecticide applications for the control of houseflies: direct insecticide application to the breeding sites for larval control (larvicides), application of residual sprays on housefly resting sites, introducing toxic man-made resting sites (impregnated cords/strips), applying toxic baits, applying space sprays directly to fly aggregations, and, last applying vapor toxicants (Keidig 1986, Barnard 2003).

Larvicides are applied as spot treatments on a regular basis in those areas where fly larvae are breeding. Some insecticides that have been used as larvicides include the organophosphates diazinon, trichlorfon, and fenthion, and several pyrethroids such as cypermethrin, deltamethrin, and permethrin. The insecticides are applied in different formulations such as emulsions or suspensions to thoroughly wet the upper 10-15 cm of the breeding medium (Barnard 2003). This method of control, however, should only be considered as an alternative to sanitation, and most of the times as a poor alternative. One of the problems that appear from the use of larvicides is the mortality of natural predators and parasites of houseflies (Keiding 1986, Scott et al. 1991). It has been suggested that even if larvicides offer temporary control they may result in increase of the fly population by disrupting the biological regulation by naturally occurring predators. Two products that have been used for fly larvae control and don't appear to have any important adverse effects on non-target organisms are the insect growth regulators diflubenzuron and cyromazine (Keiding 1986).

Treating naturally occurring resting areas of houseflies with residual insecticides or even introducing insecticide treated resting sites (such as toxicant impregnated strips and cords) is another popular approach for fly control. These are low-pressure, spot treatments of residual insecticides on those surfaces that flies are anticipated to land and rest. Several examples of insecticides that have been used for this type of application are the organophosphates (dimethoate, trichlorfon and naled), and the pyrethroids (cypermethrin, permethrin, and deltamethrin) (Barnard 2003). The effectiveness of this method depends on the type of the insecticide used, the environmental conditions like sunlight exposure which accelerates the insecticide degradation, but mostly it depends on the right location of the treatment in time and space according to the resting behavior of the fly (Keiding 1965). Keiding (1965) in his review

on observations of the housefly behavior in relation to its control concluded firstly, that houseflies show preference for resting indoors at lower night temperatures (below 15-20 ^{0}C) and outdoors in warmer nights, secondly the upward movement of flies to the ceiling or branches of trees after sunset, and last the general preference of flies to rest on narrow objects, edges, and anything protruding from large surfaces. He suggested that since houseflies tend to have an aggregated night time distribution, control efforts should be mostly directed against the night resting sites.

Insecticides in the form of baits came into prominence in the early 1950's (Gahan et al. 1953). The first form of baits that were initially used for fly control contained simple sugar water or some other type of attractant combined with poisons such as sodium arsenite and formaldehyde. Since the development of modern insecticides, newer baits have been developed that can be divided into three main categories: dry scatter baits, liquid baits, and paint-on baits (Keiding 1986, Barnard 2003). The newer baits utilize a variety of organophosphate (i.e. dimethoate, malathion, naled, diazinon) and carbamate (i.e. propoxur, bendiocarb, methomyl) insecticides as active ingredients. The effectiveness of the baits to attract flies can be enhanced by the addition of attractants, such as the sex pheromone, muscalure. Baits can provide satisfactory control and reduce fly populations in short periods of time. However, they must be applied one to six times per week (Barnard 2003) in order to be effective. Also, they have the advantage that development of resistance is generally less compared to residual sprays. They must be kept, however, away from animals and children.

Both outdoor and indoor space treatments for housefly control involve the usage of mists or aerosols of insecticide solutions or emulsions that directly target aggregations of resting or flying adults. Space treatments do not provide long-term fly control but instead they provide a

temporary relief from housefly nuisance. Therefore they should be applied in those situations where excessive housefly nuisance is being observed, and they should be used as an additional tool and not as the main approach technique for controlling houseflies. For indoor applications, hand and power sprayers are used to apply the material. For the outdoor applications mist sprayers, thermal foggers, or even Ultra Low Volume (ULV) application methods can be used to disperse the material. For indoor treatments natural pyrethrins or synthetic pyrethroids would be the insecticide of choice, due to their ability to provide quick knockdown without presenting any toxic hazards (Schmidtmann 1981). Also, indoors space treatments must be applied during those times when most flies are aggregated indoors. For the outdoor treatments the application can take place both by ground and air (Mount, 1985) and has as ultimate goal to eliminate fly populations around those areas with high human activity such as recreational areas and food markets. For the outdoor treatments, in addition to the pyrethroids, several organophosphate compounds are used as well (i.e. malathion, naled, diazinon).

One last approach for fly control involves the use of slow release vapor toxicants. This has been one of the least popular control methods and there has been little research conducted to test the effectiveness of such an application. This could be attributed to the lack of insecticidal compounds with effective vapor toxicities. Dichlorvos (DDVP) is the one insecticide mostly studied as a vapor toxicant against house flies (Miles et al. 1962, Matthysse & McClain 1972). Dichlorvos is an organophosphate insecticide and for the first time it was registered to be used as an insecticide in 1948 (EPA 2006). A very common formulation of dichlorvos is in resin strips. The resin strips were shown to work effectively against adult flies in enclosed spaces. Resin strips were, also, proven effective for fly control within garbage cans or other similar receptacles that may not be fly-tight. The high mammalian toxicity of dichlorvos, in combination to reported

resistance incidents (Bailey et al. 1971) has limited the use of dichlorvos as a fly control approach. Therefore, there is a need for new insecticidal compounds, with good vapor toxicities and novel modes of action that will replace dichlorvos. This research evaluated vapor toxicity of novel, low molecular weight, highly volatile formate, acetate, and heterobicyclic compounds on house flies.

CHAPTER 4
LITERATURE REVIEW: NOVEL VOLATILE COMPOUNDS AND INSECTICIDE SELECTIVITY

Novel Volatile Compounds

Insecticides are divided into five categories according to their mode of action: physical poisons, protoplasmic poisons, metabolic inhibitors, neuroactive agents and stomach poisons (Matsumura 1980). Some insecticides have multiple modes of actions, as that seems to be the case with the novel compounds studied in this thesis project. Nguyen et al. (2007) studied toxicity, synergism and neurological effects of the novel formates, acetates, and heterobicyclics on *Drosophila*. *Drosophila* was chosen as representative of the order Diptera. According to their findings, the compounds possess a diverse range of activities and modes of actions, as they seem to act as both metabolic inhibitors and neuroactive agents. They were able to identify a role for cytochrome P450-based metabolism in activation and/or deactivation of the various heterobicyclics, esterase-based activation of some formate esters, and finally neurological action at chloride and sodium channels by the novel compounds.

Also, Haritos & Dojchinov (2003) studied a range of alkyl esters on beetles, in an attempt to discover the toxic agent of the alkyl esters within the insects. Their intentions were to determine whether it was the intact ester or one or more of its break down products that were responsible for the toxic effects. Their findings revealed esterase-based activation of the formate esters, which comes in agreement with Nguyen et al (2007). Haritos & Dojchinov (2003) showed that volatile formate esters were more toxic than other alkyl esters due to their hydrolysis to formic acid and its inhibition of cytochrome c oxidase. The process involves 3 main steps. First, a wide variety of many esterases hydrolyse the formate esters into formic acid and their corresponding alcohols. Then, formic acid binds to cytochrome a_3 and inhibits cytochrome c oxidase activity (Nicholls 1975). Last, the inhibition of cytochrome c oxidase prevents the

utilization of molecular oxygen by cells, leading to loss of cell function and subsequently cell death.

This research evaluated the vapor toxicity effects of the novel volatile compounds on two different insect species: the yellow fever mosquito and the common house fly. There is no work published to my knowledge regarding the mode of action of the novel volatile esters and heterobicyclcis on mosquitoes and house flies. This paper constitutes the first publication on the toxicity of the novel volatile compounds on mosquitoes and house flies.

It is very often that insecticides exhibit different toxicities among different insect species (Camp at al. 1969, Coats 1979, Mallipudi & Fukuto 1979). Understanding how various insecticides exhibit different toxicities among different insect species (insecticide selectivity) will be necessary in order to appropriately explain and discuss the results presented in Chapters 5 & 6 of this paper.

Insecticide Selectivity

Once the insecticide enters the insect body it is recognized as a foreign substance or "xenobiotic", and is metabolized to a less toxic and more polar substance that will eventually be removed from the body. This metabolic process is called "detoxicification". However, it has been shown that insecticides can also be converted into more toxic substances once within the insect body. This process is known as "activation" (Feyereisen 2005). By far the two most significant reactions involving the metabolism of insecticides are the NADPH-requiring cytochrome P450 mono-oxygenases and the esterases or hydrolases (Feyereisen 2005, Oakeshott et al. 2005). The first system is also known as the "mixed function oxidase" (MFO) system and it performs the first oxidative enzymatic attack on xenobiotic compounds. These enzymes are quite versatile and accept most xenobiotic copmpounds as their substrate. They require NADPH to deliver the electrons down an electron transport system with cytochrome P450 as the terminal

oxidase of the electron transport chain. The final product of this reaction is the oxidized form of the xenobiotic compound. The second reaction is a hydrolysis reaction and it involves the action of several hydrolases, such as carboxylesterases, amidases, type A-esterases, which split esteratic insecticide substrates with the addition of water to yield alcohols and acids as the final products.

The activity of these two enzymatic systems varies among different insect species, potentially resulting in species differences in susceptibility to various insecticidal compounds. Also, both enzymatic systems have been involved in insecticide resistance mechanisms. Following I have several examples that demonstrate how the activities of these 2 enzymatic systems vary among different insects and can affect the insect responses on various insecticides. Casida et al. (1976) reported different ability of esterases to hydrolize pyrethroid insecticides among 5 different insect species. Brooks (1986) reported esterases to be more important enzymes for pyrethroid detoxification in *Spodoptera littoralis* (the Egyptian cotton leafworm), *Trichoplusia ni* (cabbage looper) and *Chrysoperla carnea* (common green lacewing) larvae, and oxidases more important in *Tribolium castaneum* (red flour beetle) larvae. Claudianos et al. (2006) reported that honeybee shows much greater susceptibility to insecticides compared to *Anopheles gambiae* and *Drosophila* due to a deficit of detoxification enzymes: there are only about half as many cytochrome P450 monooxygenases and carboxyl/cholinesterases in the honeybee compared to *Anopheles gambiae* and *Drosophila*. Phillips at al. (1990) and Benedict et al. (1994) showed that genetically transformed Drosophila (op degrading gene) with high levels of organophosphate hydrolases shows over 20-fold greater paraoxon resistance compared to untransformed controls. Chang & Whalon (1987) showed that in resistant strains of predatory mites some esterase isozymes demonstrated higher rates of synthetic pyrethroid hydrolysis compared to the non-resistant strains. Also, P450 over expression was shown to various

insecticide resistant strains. For example Kasai et al. (1998) showed that the metabolism of permethrin to 4-hydroxypermethrin was higher in microsomes from Culex mosquito larvae resistant to permethrin than from the susceptible strain. Last, conversion of fipronil to its sulfone by P450 has a marginal effect on the toxicity of the parent chemical in Diabrotica virgifera (Scharf et al. 2000). However, in Blattela germanica it was shown that the oxidation of fipronil to its sulfone constitutes an activation step (Valles et al. 1997).

CHAPTER 5
EVALUATION OF VAPOR TOXICITY OF NOVEL LOW MOLECULAR WEIGHT COMPOUNDS ON MOSQUITOES

Introduction

Volatile insecticides have been commonly used as fumigants for the control of structural pests and the protection of agricultural commodities. However, they have been mostly ignored for the control of medical importance pests such as mosquitoes and flies. Dichlorvos (DDVP) is the one volatile insecticide studied mostly on mosquitoes and flies. Dichlorvos is an organophosphate insecticide and was registered in 1948 (EPA 2006). One very common formulation of dichlorvos is resin strips. Resin strips were initially registered for use in areas where flies, mosquitoes and other nuisance pests occur. Dichlorvos has been classified by the Environmental Protection Agency (EPA) as a "probable human carcinogen", and because of its implications in human health in 2006, its use in homes was restricted to confined spaces such as wardrobes, cupboards and closets (EPA Office 2006). Therefore, there is a need for replacement of dichlorvos with friendlier, less toxic chemistries. Highly volatile, low molecular weight formates, acetates, and heterobicyclics may be potential replacements for dichlorvos, and in their own right may offer a new class of chemistry.

Thirty novel, low molecular weight compounds with insecticidal activity were tested on *Drosophila melanogaster* Meig. (Scharf et al. 2006). The compounds belonged to six different families: heterobicyclics, formates, acetates, propionates, butyrates and valerates. *Drosophila* was used as a model to assess potential efficacy of these novel chemistries against mosquitoes and flies. Findings showed 7 highly effective compounds with vapor toxicity: four formate esters and three heterobicyclics. The reaction of an organic acid and an alcohol is called esterification, where the end products are always ester and water. Formate esters are organic compounds composed of formic acid and a corresponding alcohol. Acetate esters, similarly to formate esters,

are composed of acetic acid and a corresponding alcohol. On the other hand the structure of heterobicyclics is made from fused 5, 6-membered rings.

For my research I investigated the vapor toxicity effect of 4 heterobicyclic compounds, 7 formate, and 4 acetate esters directly on mosquitoes. Most of the compounds that I evaluated in the work presented here are naturally occurring products. They are found on fruits such as apples, bananas, strawberries, oranges, kumquats, and coconuts just to name a few. They are commercially used as flavoring agents in products such as coffee, chocolate, fruity drinks, rum, wine, and tobacco. Most of the compounds have a rather strong and fruity odor, and therefore they have many uses as odor agents. Another interesting characteristic of these products is that they are part of the chemical structure of some pharmaceutical drugs, responsible for treating insomnia, osteoporosis, and asthma. In Tables 5-1, 5-2, and 5-3 the chemical structures of each individual chemical can be seen. Information such as molecular weight, boiling point, density, natural occurrence and other physical properties are included in the same table as well.

Materials and Methods

Chemicals

Fifteen novel insecticides (Sigma Aldrich Chemical, Milwaukee, WI) were tested; 7 formate esters [ethylene glycol di-formate (EGDF), methyl formate, ethyl formate, propyl formate, butyl formate, hexyl formate and heptyl formate), 4 heterobicyclic esters (menthofuran, benzothiophene, coumaran and dimethyl-coumarone) and 4 acetate esters (propyl acetate, butyl acetate, pentyl acetate and hexyl acetate). Dichlorvos (DDVP) was tested as a positive control (Chem Service, West Chester, PA). All insecticides were >99% pure and in liquid form except for thiophene that came in a crystalline solid form. Insecticide stock solutions were prepared in acetone at concentrations of 2, 100, 150, 200, 300 and 400 µg/µl. All compounds and stock

solutions were held at –20°C in glass vials with rubber lined caps to prevent vapor escape, until placed in experiments.

The insecticide synergists SSS-tributyl-phosphorotrithioate (DEF) and piperonyl butoxide (PBO), which are esterase and cytochrome P450 inhibitors respectively, were used (Mobay Chemical Co., Kansas City, MO and MGK Inc., Minneapolis, MN). DEF and PBO were >95% pure. DEF and PBO stock solutions were prepared at 100 µg/ml in acetone.

Insects

Mosquitoes [USDA-CMAVE Orlando strain of *Aedes aegypti* (L.)] reared at the University of Florida in Gainesville were used. Mosquitoes were reared on a 12:12 (L: D) photoperiod, at 25°C and ~50% RH. Mosquito larvae were fed on a powder diet consisting of 2 parts liver (MB Biomedicals LLC, Aurora, OH) and 3 parts yeast (Modern Products Inc., Thiensville, WI). The diet was diluted in deionized water to a 40 g/liter concentration. Approximately 1,500 larvae were reared in plastic trays (53.3 by 40.6 cm) containing 3 liters of water. The quantity of the diluted diet varied depending on the larval instar. Mosquito larvae were not fed for 24 h after hatching. Second and third instars were fed 30 ml of the diluted medium per day; whereas, the diet of the fourth instars was decreased to 20 ml per day. When majority of pupation had occurred no more food was provided. Pupae were removed and placed into deli cups filled with deionized water. The deli cups were then placed into screened rearing cages (39.4 by 26.7 by 26.7 cm) for adult emergence. Mosquito adults were maintained on a 10% [w/v] solution of sugar water.

Prior to each treatment 3 to 5-d-old adult mosquitoes were aspirated from their cages and placed into plastic deli cups on ice until their activity was reduced. Ten females were removed from the deli cups using a feather tip forceps. A minimum of 300 mosquitoes were selected for exposure to each insecticide.

Bioassay

Main bioassay set-up. This bioassay was adapted from Scharf et al. (2006) and Nguyen et al. (2007) (Fig. 5-8). Ten females were transferred from the deli caps into 125 ml plastic vials. Caps with an opening of ~2.6 cm in diameter, covered with common fiberglass window screening (~1.55 mm mesh), were used to close the vials. The screening prevented insect escape while allowing for gas exchange. Along with the mosquitoes a cotton wick (~1.5 cm in length) dipped in 10% w/v solution of sugar water was placed in the vials. A toothpick (~6.3 cm in length) was used to support the wick. The wick was provided as the nutrient and moisture source. The mosquitoes were given 1 h to recover from the chilling effects of the ice prior to the treatment, and then every vial was placed into a Mason 1 liter (1 quart) glass jar along with an untreated filter paper (55 mm in diameter). Prior to closing the glass jar the filter paper was treated with the proper quantity of insecticidal solution using an eppendorf pipette. The concentrations of the insecticide solutions varied depending on the insecticide tested. Methyl and propyl formate were applied at a 100 µg/µl concentration and in a range from 1.2-1.8 mg. Coumaran, butyl formate, and hexyl formate were applied at a 150 µg/µl concentration and in a range from 0.75-3 mg, 1.05-1.95 mg, and 1.05-1.95 mg, respectively. Menthofuran, benzothiophene, ethyl formate, heptyl formate, EGDF, propyl acetate and butyl acetate were applied at a 200 µg/µl concentration and in a range from 2-4 mg, 1.6-4 mg, 1.4-2.6 mg, 1.6-4 mg, 2-4 mg, 2.8-4 mg and 2.8-4 mg, respectively. Dimethyl-coumarone and hexyl acetate were applied at a 400 µg/µl concentration and in a range from 2-8 mg. DDVP was applied at a 2 µg/µl concentration and in a range from 0.016-0.04 mg. There was, also, a blank control where the filter paper received no chemical at all, and a solvent control, which received a volume of acetone identical to the highest insecticide solution volume (up to 20 µl). The jars were closed rapidly and tightly to prevent vapor escape and after a 24 h exposure mortality was recorded. In

order to determine mortality the jars were shaken for a minimum of 15 sec before mosquito movement was observed. A mosquito was recorded dead when there was no movement observed.

Synergist (DEF and PBO) bioassay set-up. The effect of the synergists PBO and DEF for toxicity was investigated on three of the fifteen insecticides tested above: ethylene glycol di-formate, heptyl formate and menthofuran. The synergist bioassays were conducted in the same way described above except that an extra step was added. That extra step involved the exposure of the mosquitoes to the synergist, prior to their exposure to the insecticides (Nguyen et al. 2007). For the synergist bioassay the plastic vials were replaced with 125 ml glass vials to prevent absorption of the synergist into the plastic. Synergist stock solutions at 100 µl were pipetted to every glass vial, using an eppendorf pipette, so that every vial would contain 10 µg of the synergist. Previous studies have shown that this synergist quantity causes no mortality in Drosophila after 24 h of exposure (Nguyen et al. 2007). After treating the vials with the synergist the vials were rolled on their sides under a fume hood to ensure equal distribution of the synergist on the inner surfaces while the acetone evaporated. Once acetone evaporated, 10 mosquitoes were added in every glass vial along with a moist cotton wick, and were held for an hour to allow for the synergist to take effect. Along with the blank and the solvent control, a synergist control was added where the mosquitoes were only exposed to the synergist.

Data Analysis

In those cases where control mortality was observed data was adjusted using the Abbott's formula (Abbott 1925). When control mortality exceeded 10% that rep was discarded. Probit analysis was performed and the LC_{50} and LC_{90} of each insecticide with and without the synergist were estimated (SAS Institute 2003). The data reported in Tables 5-4, and 5-5 include slope, goodness of fit characteristics (chi-square, P-value) and LC_{50} and LC_{90} estimates with 95%

confidence limits. LC estimates with non overlapping 95% confidence limits were considered significantly different.

Body-weight corrected LC_{50s} of each insecticide for mosquitoes and *Drosophila* were calculated (Table 5-6, Fig. 5-7). One hundred individuals from both species were weighed and that weight was recorded. That number was then divided by 10 to give the average weight of 10 mosquitoes and 10 *Drosophila* (0.0153 and 0.006 g, respectively). The average weight of the 10 insects was used to adjust the LC_{50} from mg/liter into mg/ g of insect body weight/liter.

PoloPlus 2.0 (2005) was used to calculate the potency ratios of the LC50s with & without the synergist. The program calculated 95% confidence limits for every ratio. The 95% CI were used to determine whether there were significant differences in the LC50s due to the effect of the synergists (Table 5-5).

Linear regression analyses were performed (SAS Institute 2003) that compared LC_{50} estimates versus molecular weight, density and boiling point of the seven formate esters, the four heterobicyclics and the four acetate esters (Figs. 5-3, 5-4, 5-5, and 5-6).

Results

Toxicity Evaluation of Novel Compounds

DDVP was by far the most toxic compound tested on mosquitoes. Specifically, it was 54.4 times more toxic compared to the second best compound, the formate ester methyl formate. Within the novel compounds, overall, formate esters were the most toxic family followed by the heterobicyclics and, last, by the acetate esters (Table 5-4, Fig. 5-1).

Formate esters. Methyl formate was the most toxic ester (LC_{50} estimate 1.36 mg/liter), followed by butyl, propyl, ethyl, hexyl formate, EGDF, and heptyl formate. The toxicities of propyl and ethyl formate were not significantly different and there was no major difference between them and butyl formate. EGDF and heptyl formate (LC_{50} estimates 2.99 and 3.17

mg/liter, respectively) were the least toxic formate esters with toxicities in the same range as the heterobicyclics, the second best performing family of esters.

Heterobicyclics. Coumaran was the most toxic heterobicyclic (LC_{50} estimate 2.03 mg/liter), followed by benzothiophene, dimethyl-coumarone and menthofuran. Benzothiophene and dimethyl-coumarone were not significantly different. There were significant differences in the slopes among the 4 heterobicyclic compounds. Coumaran, the best performing heterobicyclic, had the smallest slope, which suggests that there is a lot of heterogeneity on the response of the insects to the insecticide. On the other hand, menthofuran, the heterobicyclic with the poorest performance, had the biggest slope, which suggests a lot of homogeneity on the response of the insects towards the insecticide.

Acetate esters. Hexyl acetate was the least toxic compound (LC_{50} estimate 5.09 mg/liter). The toxicities of propyl, butyl and pentyl acetate were not significantly different.

Toxicity Evaluation of Novel Compounds with the Synergistic Effect of DEF and PBO

Whne heptyl formate and EGDF were cotreated with DEF their toxicities decreased significantly (Table 5-5, Fig. 5-2). The toxicity of heptyl formate decreased by 1.35 times, where as the toxicity of EGDF decreased by 2.56 times. Also, when menthofuran was combined with PBO, its toxicity increased significantly.

Evaluation of the Role of Volatility in Toxicity

Molecular weight, density, and boiling point were investigated as predictors of volatility. A chemical with lower molecular weight (lighter chemical), lower boiling point and lower density volatilizes faster compared with a chemical having higher molecular weight (heavier chemical), higher boiling point and higher density. Regression analysis between toxicity and volatility predictors was performed for each of the three families separately and for all three families together (Figs. 5-3, 5-4, 5-5, and 5-6). For the formate esters the regressions of LC_{50} versus

molecular weight, boiling point, and density were correlated with R^2 0.57, 0.69, and 0.19, respectively. For the heterobicyclics the regression of LC_{50} versus molecular weight was correlated with R^2 0.88. On the other hand, the regressions of LC_{50} versus density and boiling point were weak ($R^2 < 0.25$). Last, for the acetate esters the regressions of LC_{50} versus molecular weight, density and boiling point were weak ($R^2 = 0.24$, $R^2 = 0.20$, $R^2 = 0.24$ respectively). When combined families regression was performed there was a poor correlation between toxicity and all 3 volatility predictors, except maybe with molecular weight ($R^2 = 0.5$).

Discussion

Comparing Toxicities of Novel Compounds Among Mosquitoes and *Drosophila*

My study is the first report of the toxic effects of the novel volatile formate, heterobicyclic, and acetate compounds on mosquitoes. Scharf et al. (2006) reported the toxicity of these novel compounds on *D. melanogaster* Meig. *Drospophila* was used as a model to assess potential efficacy of these compounds on mosquitoes and flies. Body-weight corrected LC_{50} values (in mg per g of insect per liter) of the 15 volatile compounds and the organophosphate DDVP on mosquitoes and *Drosophila* can be seen in Table 5-6 and Fig. 5-7. There were significant differences among the toxicities of the compounds on mosquitoes and *Drosophila*. DDVP was by far the most toxic insecticide for both insects and was significantly more toxic to mosquitoes than *Drosophila*. DDVP has been known as a very effective insecticide against various insects for many years. Maddock and Sedlack (1961) gave one of the earliest reports regarding the toxicity of DDVP on mosquitoes. They reported that 0.015 µg of DDVP per liter of air will give 100% kill of *Anopheles* mosquitoes. All compounds, except for menthofuran, were significantly more toxic to mosquitoes than *Drosophila*. On average the 14 compounds were approximately 3.5 times more toxic to mosquitoes, whereas menthofuran was 1.7 times more toxic to *Drosophila*. On mosquitoes there was a toxicity trend observed among the three families, with

formates showing overall higher toxicity, followed by heterobicyclics and last by acetates. However, there was not an apparent trend on toxicity among the three ester families on *Drosophila*. Some of the best performing compounds and some of the poorest ones belonged to the same chemical families. It was only the acetate esters that consistently showed poor toxicity. The best 7 performing insecticides with vapor toxicity on *Drosophila* were the two heterobicyclics menthofuran and benzothiophene. These two compounds were followed by the formate esters butyl, hexyl, heptyl formate, the heterobicyclic coumaran and the formate ester ethyl formate. The best 7 performing compounds on mosquitoes were the formate esters methyl, butyl, propyl and ethyl formate. These compounds were followed by the formate ester hexyl formate, the heterobicyclics coumaran, and benzothiophene. What is interesting is that the most toxic compound on *Drosophila,* menthofuran, is one of the least toxic compounds when tested on mosquitoes. Conversely, the most toxic compound on mosquitoes, methyl formate, is one of two least toxic compounds when tested on *Drosophila*.

There are several possible explanations as to why the compounds performed differently on mosquitoes than *Drosophila*. A first explanation could be differences on the insect handling techniques during the experimentation. Scharf et al. (2006) used CO_2 to knock down *Drosophila* prior to exposing them on the insecticides, whereas I used ice for knocking down mosquitoes. Another explanation could be physiological differences in acquiring and metabolizing insecticides. The lethal effects of insecticidal compounds depend upon the amount of insecticide that reaches the target site (site of action). The amount of insecticide that reaches the target site is controlled by certain processes such as penetration through the insect cuticle, diffusion through the insect spiracles, bioactivation/biodegradation within the insect body, travel distance to the site of action and finally excretion just to name a few (Quraishi 1977, Matsumura 1980, Yu

2006). Different insect species can differ greatly in susceptibility to insecticidal compounds due to distinct differences in the physical and physiological processes mentioned above. Insecticides with high vapor pressures, such as the insecticides studied in this paper, show the tendency to enter the insect body through the spiracles (Matsumura 1980). Therefore, the susceptibility of an insect to a vapor toxicant is believed to be correlated with its rate of respiration (Vincent & Lindgren 1965). In general, different insects exhibit different patterns of gas exchange when at rest (Lighton 1988, 1990, Lighton & Berrigan 1995). The most familiar and well studied pattern is the Discontinuous Gas Exchange Cycle (DGC) (Kestler 1985, Lighton 1994), where the spiracles remain closed for lengthy periods of time allowing for no gas exchange. This closed-spiracle phase is followed by a fluttering-spiracle phase and finally an open-spiracle phase where the accumulated CO_2 escapes form the tracheal system to the surrounding environment. Little information, however, is available on the respiratory pattern of small insects (body weight ~ 1 mg) such as *Drosophila* (Williams et al.1997, Williams and Bradley 1998, Lehman et al. 2000, Fielden et al. 2001), and even less information is available on mosquitoes (Diarra et al. 1999, Gray & Bradley 2003, Gray & Bradley 2006). What is known so far is that both mosquitoes & *Drosophila* have the ability to control gas release from their tracheal system. There is some evidence to support that both insects perform DGC, however further research is needed to conclusively test this hypothesis. Due to the absence of evidence one should consider that mosquitoes and *Drosophila* may follow a different breathing pattern. If this is true, the insects may be allowing different amounts of insecticide to enter their body and this may be one of the factors responsible for the different responses they show to the various insecticides tested.

Another explanation could be differences in the detoxification systems among mosquitoes and *Drosophila*. Once an insecticide enters the insect body it is perceived as a foreign substance

or xenobiotic, and is metabolized by different metabolical processes with the ultimate goal to be converted into a less toxic polar substance that will eventually be removed form the body. This metabolic process is called “detoxification”. By far the two most significant reactions involving the metabolism of insecticides are the NADPH-requiring general oxidation system and the hydrolysis of esters (Matsumura 1980). The activity of these two enzymatic systems varies among different insect species, resulting in species differences in susceptibility to various insecticidal compounds (Casida et al. 1976. Brooks 1986, Valles et al. 1997, Scharf et al. 2000).

Implications of the Synergistic Effects of PBO and DEF on the Toxicity of the Novel Compounds on Mosquitoes

The modes of action of the novel formate, acetate, and heterobicyclic compounds on mosquitoes so far remain undefined. According to the results presented in this study there seems to be a significant effect of cytochrome P450 enzymes on menthofuran detoxification. Also, there was evidence supporting esterase-based activation of both heptyl formate and ethylene glycol di-formate. When heptyl formate and ethylene glycol di-formate were synergized with the esterase inhibitor DEF their toxicities decreased by 1.35 and 2.56 times, respectively. The first finding comes in agreement with Nguyen (2007), who showed in Drosophila that P450 enzymes play a significant role in menthofuran detoxification and activation, depending on the fly strain. The second finding comes in agreement with both Haritos & Dojchinov (2003), and Nguyen (2007), who supported esterase based activation of some formate esters. In order for more legitimate conclusions to be made more extensive and complete research needs to be conducted where all of the esters will be tested in combination with both synergists on susceptible and even resistant mosquito species.

Structure-activity Relationships of the Three Families of Novel Compounds

As one might expect the tendency of a chemical to volatilize should play an important role in its vapor-phase toxicity. However, this did not always seem to be the case with the novel, volatile compounds studied in this research. Scharf et al (2006) did a combined ester and heterobicyclic regression analysis between toxicity and the three volatility predictors: molecular weight, boiling point, and density. According to their findings there was a statistically weak correlation between toxicity and all three volatility predictors. I performed regression analyses for each of the three families separately and for all of them combined together. When studied each family separately I was able to show reasonable correlation between toxicity and volatility predictors for some of the families. For the formate esters the regression analysis demonstrated a reasonable correlation between toxicity and the volatility predictors: molecular weight, and boiling point. However, there was a weak correlation between toxicity and density. For the heterobicyclics there was a strong correlation between toxicity and molecular weight, and a weak correlation between toxicity and the rest two volatility predictors (boiling point, density). For the acetate esters there was overall a weak correlation between the three volatility predictors and toxicity. When I evaluated all the compounds together there was overall a statistically weak correlation between toxicity and volatility. My findings, as well as Scharf's et al. (2006) findings, showed that high ester volatility did not necessarily coincide with high ester toxicity. What that implies is that there should be other factors, such as structure dependent factors, affecting the widely varying toxicity of the volatile esters.

With respect to the heterobicyclics 2 structure-activity relationship trends are apparent. First, when no peripheral methyl groups are present, oxygen in the first position of the furan ring is associated with greater toxicity than if sulfur is in this position (i.e. coumaran > benzothiophene). However, that contradicts Scharf's et al (2006) findings, where they showed

that sulfur in the first position of the furan ring is associated with higher toxicity. Second, when oxygen is in the first position of the furan ring and peripheral methyl branches are present, adjacent methyl branches are associated with greater toxicity than opposing methyl branches (i.e. dimethyl-coumarone > menthofuran). This finding comes in disagreement with Scharf et al. (2006), who showed that opposing methyl branches are associated with higher toxicity than adjacent methyl branches. The compounds showed different structure-activity relationships between mosquitoes and *Drosophila*, which suggests that the compounds may follow different metabolic pathways and may exhibit different modes of action within the two insect species.

With respect to the formate and acetate esters some structure activity relationships are apparent as well. First, as the aliphatic chain length on the acid group increases toxicity decreases for the majority of the formates (i.e. methyl formate>ethyl formate=propyl formate>hexyl formate>heptyl formate). On the other hand, there was a different activity-structure trend for formates when tested on *Drosophila* (Scharf et al. 2006). They showed that esters of intermediate chain length demonstrated greater toxicity (i.e butyl formate, hexyl formate), with lower toxicity for methyl and ethyl formates. Also, formates elicited higher toxicity than acetates implying that the formate group within the aliphatic chain is correlated with higher toxicity than the acetate group. This comes in agreement with Scharf et al. (2006), who showed that acetate esters were a less toxic family compared to formate esters.

In conclusion, DDVP was by far the most toxic insecticide for both insects and was significantly more toxic to mosquitoes than *Drosophila*. All insecticidal compounds, except for menthofuran, were significantly more toxic to mosquitoes than *Drosophila*. On mosquitoes there was a toxicity trend observed among the three families, with formates showing overall higher

toxicity, followed by heterobicyclics and last by acetates. The novel compound with the highest insecticide activity on mosquitoes was methyl formate.

Table 5-1. Physical and chemical properties of formate esters

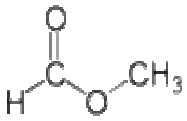
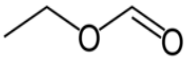
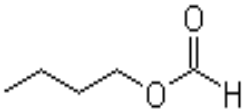
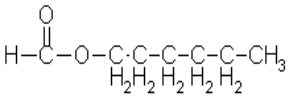
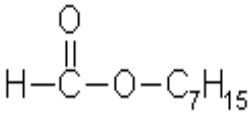
Formate Esters	Mol. Weight	bp (^{0}C)	Density (g/ml)	Natural occurrence	Used as	Other Properties
Methyl formate 	60.05	33	0.974	–	-Quick drying finishes -Alternative to sulfur dioxide in domestic refrigerators	Clear liquid with an ethereal odor
Ethyl formate 	74.08	53	0.921	–	Flavoring agent (raspberries flavor)	Characteristi c smell of rum
Propyl formate 	88.11	80.5	0.904	Apple, Pineapple, Plum, Currant	Flavoring agent(brandy & rum products)	Colorless liquid with a sweet fruity/berry odor
Butyl formate 	102.13	106.5	0.892	Pear	Flavoring/odor agent (rum, pear, plum products)	Colorless liquid with a fruity/green odor
Hexyl formate 	130.18	155.5	0.879	Pear	Flavoring/odor agent (apple, banana, lemon, strawberry, orange products)	Colorless liquid with a medium fruity odor
Heptyl formate 	144.21	178	0.882	Kumquat	Flavoring/odor agent (apple, apricot, coconut, kumquat,peach , rose, wine products)	Colorless liquid with a medium green/floral/ apple scent
Ethylene glycol di-formate 	118.09	176	1.226	–	–	Colorless, odorless liquid

Table 5-2. Physical and chemical properties of heterobicyclics

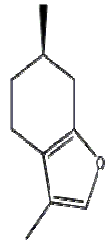
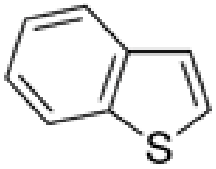
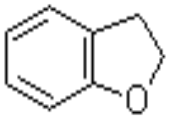
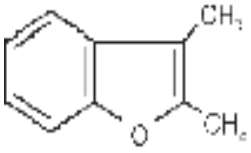
Heterobicyclic Esters	Mol. Weight	bp (^{0}C)	Density (g/ml)	Natural occurrence	Used as	Other Properties
Menthofuran 	150.22	205	0.97	Peppermint oil	Flavoring/odor agent (chocolate, coffee, peppermint)	Bluish clear liquid with a musty nutty/coffee odor
benzothiophene 	134.20	221.5	1.149	Constituent of petroleum related deposits (lignite tar)	Found in the chemical structure of pharmaceutical drugs for treating osteoporosis & asthma (raloxifen, zileuton)	Solid crystalline form with an odor similar to naphthalene
Coumaran 	120.15	188.5	1.065	_	Found in the chemical structure of pharmaceutical drugs (insomnia treatments)	_
Dimethyl-coumarone 	146.19	101.5	1.034	Cade oil Tobacco Coffee	Flavoring/odor agent (chocolate, coffee, tobacco, vanilla, leather products)	Pale yellow liquid with a strong phenolic odor

Table 5-3. Physical and chemical properties of acetate esters

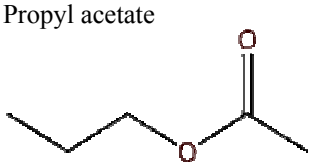
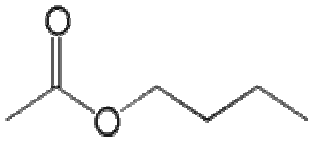
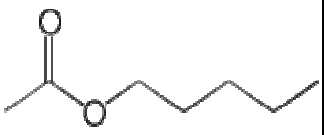
Acetate Esters	Mol. Weight	bp (^{0}C)	Density (g/ml)	Natural occurrence	Used as	Other Properties
Propyl acetate 	102.3	102	0.888	_	Flavoring/odor agent	Clear colorless liquid with an odor of pears
Butyl acetate 	116.16	125	0.88	Several fuits (eg. Apples in the Red Delicious variety)	Flavoring agent (candy, ice-cream, cheeses, baked goods)	Colorless liquid with a fruity odor
Pentyl acetate 	130.18	146	0.876	_	_	Colorless liquid with an odor similar to banana odor
Hexyl acetate 	144.21	169	0.87	_	Flavoring and fragrance agent	Colorless liquid with a fruity/pear odor

Table 5-4. Vapor toxicities of 15 novel, low molecular weight, volatile compounds and the organophosphate DDVP to mosquitoes *Aedes aegypti* (L.)

Insecticide Families Insecticides	Slope ± SE	LC50 mg/liter (95% CI)	LC90 mg/liter (95% CI)	$\chi 2$	P
Organophosphates					
DDVPa	4.84 ± 0.51	0.025 (0.023-0.027)	0.047 (0.042-0.056)	4.50	0.11
Formate esters					
Methyl formate	9.84 ± 1.10	1.36 (1.311-1.40)	1.83 (1.74-1.98)	4.09	0.13
Ethyl formate	9.12 ± 0.82	1.7 (1.64-1.78)	2.37 (2.25-2.54)	3.06	0.22
Propyl formate	7.87 ± 1.70	1.69 (1.62-1.80)	2.45 (2.15-3.38)	2.66	0.10
Butyl formate	7.79 ± 0.76	1.54 (1.48-1.60)	2.25 (2.08-2.50)	4.50	0.10
Hexyl formate	7.52 ± 0.90	1.86 (1.77-2.00)	2.76 (2.47-3.29)	4.00	0.13
Heptyl formate	4.79 ± 0.55	3.17 (2.92-3.51)	5.88 (4.99-7.54)	4.56	0.33
EGDF	9.12 ± 0.81	2.99 (2.89-3.11)	4.14 (3.90-4.48)	1.98	0.96
Heterobicyclics					
Menthofuran	11.66 ± 1.72	3.62 (3.51-3.73)	4.66 (4.37-5.21)	2.36	0.49
Benzothiophene	4.83 ± 0.50	2.89 (2.71-3.10)	5.33 (4.70-6.42)	1.20	0.54
Dimethyl-coumarone	7.86 ± 0.49	2.98 (2.88-3.09)	4.35 (4.13-4.62)	4.94	0.55
Coumaran	3.14 ± 0.34	2.03 (1.84-2.26)	5.19 (4.22-7.05)	2.57	0.27
Acetate esters					
Propyl acetate	5.89 ± 1.22	4.31 (3.98-5.11)	7.11 (5.73-11.8)	0.24	0.88
Butyl acetate	7.83 ± 1.22	3.91 (3.73-4.21)	5.70 (5.04-7.16)	0.03	0.98
Pentyl acetate	8.04 ± 1.21	3.80 (3.65-4.05)	5.49 (4.91-6.72)	0.24	0.88
Hexyl acetate	6.15 ± 0.69	5.09 (4.75-5.41)	8.23 (7.52-9.38)	0.63	0.42

[a] Positive Control.

Table 5-5. Vapor toxicity of EGDF, heptyl formate & menthofuran with and without the synergistic effect of DEF and PBO to mosquitoes *Aedes aegytpi* (L.)

Insecticides	Slope ± SE	LC50 mg/liter (95%CI)	LC90 mg/liter (95% CI)	$\chi 2$	P	Potency ratio a
EGDF	9.12 ± 0.81	2.99 (2.89-3.11)	4.14 (3.90-4.48)	1.98	0.96	
EGDF + DEF	6.36 ± 0.69	7.67 (7.23-8.08)	12.19 (11.19-13.80)	0.34	0.98	2.56 (2.39-2.73)
Heptyl formate	4.79 ± 0.55	3.17 (2.92-3.51)	5.88 (4.99-7.54)	4.56	0.33	
Heptyl formate + DEF	8.33 ± 0.82	4.29 (4.12-4.47)	6.12 (5.74-6.70)	2.20	0.69	1.35 (1.23-1.49)
Menthofuran	11.66 ± 1.72	3.62 (3.51-3.73)	4.66 (4.37-5.21)	2.37	0.49	
Menthofuran + PBO	8.47 ± 0.96	3.37 (3.24-3.52)	4.77 (4.39-5.42)	5.37	0.14	0.932 (0.89-0.98)

[a] LC_{50}+synergist / LC_{50}.

Table 5-6. Body-weight corrected vapor toxicities of 15 novel, low molecular weight, volatile compounds and the organophosphate DDVP to mosquitoes *Aedes aegytpi* (L.) and *Drosophila melanogaster* Meig.

Insecticide Families Insecticides	Mosquito LC50 mg/gr of insect/liter (95%CI)	Drosophila LC50 mg/gr of insect/liter (95%CI) c
Organophosphates		
DDVPa	1.68 (1.5-1.76)	3.7 (3.2-4.3)
Formates esters		
Methyl formate	88 (85.68-91.5)	824 (636.6-1,776)
Ethyl formate	112 (107.2-116.3)	550 (493.3-636.6)
Propyl formate	110 (105.8-117.6)	610 (593-626.6)
Butyl formate	100 (96.7-104.5)	304 (266.6-332)
Hexyl formate	130 (115.6-130.7)	380 (356.6-400)
Heptyl formate	206 (190.8-229.4)	450 (420-475.3)
EGDF	194 (188.8-203.3)	834 (676.6-1,846)
Heterobicyclics		
Menthofuran	230 (229.4-243.7)	136 (120-150)
Benzothiophene	190 (177.1-202.6)	266 (236.6-293.3)
Dimethyl coumarone	194 (188.2-201.9)	654 (513.3-817.3)
Coumaran	132 (120.3-147.7)	490 (446.6-553.3)
Acetate esters		
Propyl acetate	282 (260.1-333.9)	683 (ND)b
Butyl acetate	256 (243.8-275.2)	607 (576.6-643.3)
Pentyl acetate	248 (238.5-264.7)	597 (550-660)
Hexyl acetate	333 (310.4-353.6)	553 (526.6-583.3)

[a] Positive control.
[b] Not determined.
[c] *Drosophila* data Scharf et al. 2006.

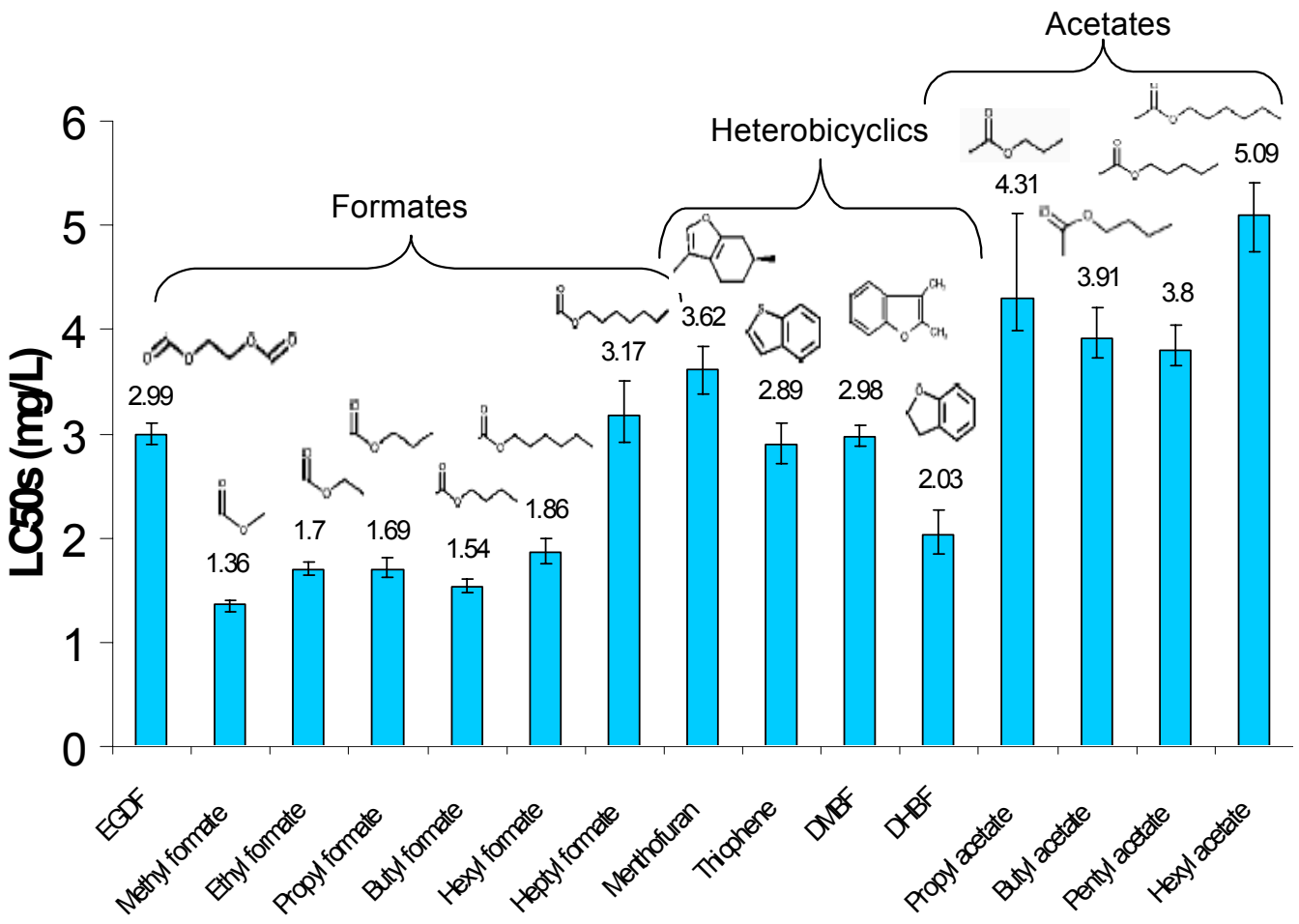


Figure 5-1. The LC_{50} values of mosquitoes *Aedes aegypti* (L.) when exposed on vapors of 15, novel, low molecular weight compounds.

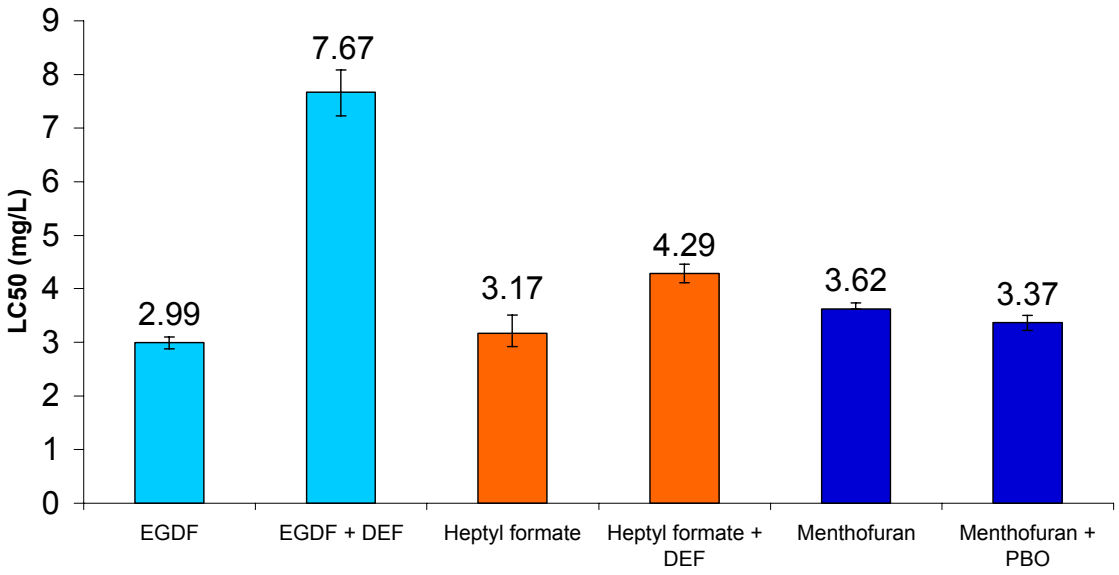
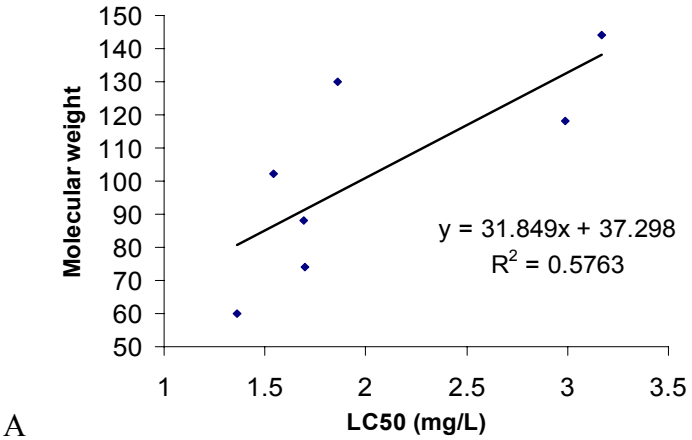


Figure 5-2. The LC_{50} values of mosquitoes *Aedes aegypti* (L.) when exposed on the vapors of EGDF, heptyl formate, and menthofuran with and without the synergistic effect of DEF and PBO.



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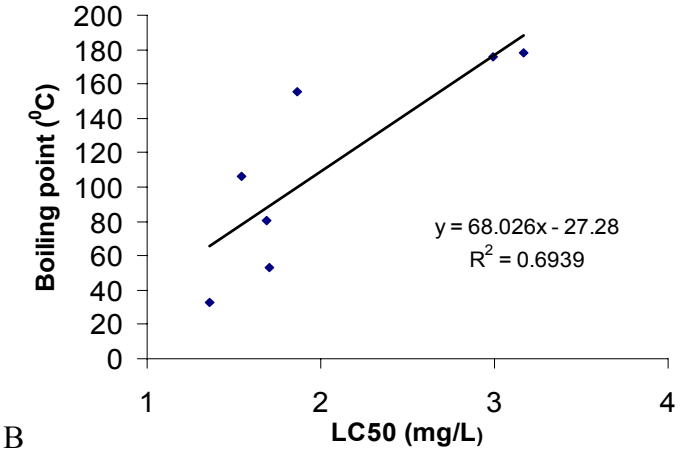

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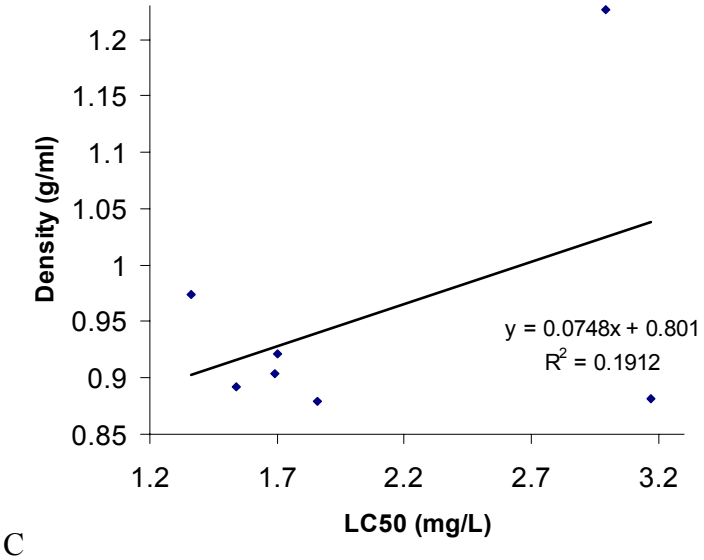
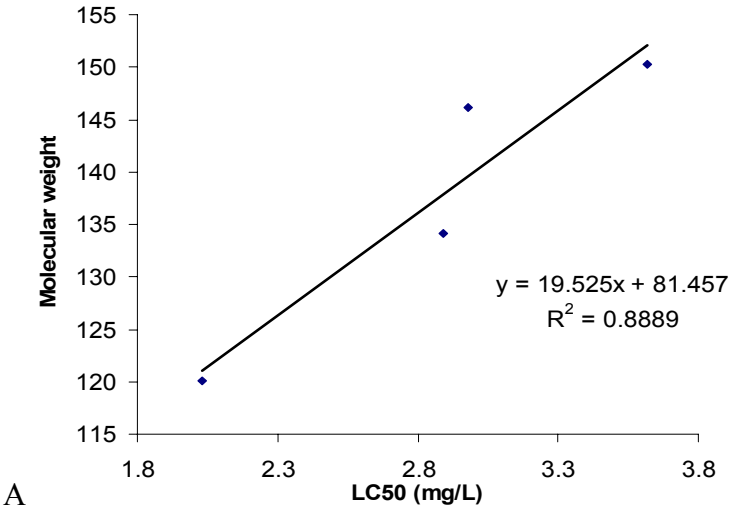


C

Figure 5-3. Regression analyses of the LC_{50} versus the physical properties of each of the 7 formate esters. A) LC_{50} versus molecular weight. B) LC_{50} versus boiling point. C) LC_{50} versus density.



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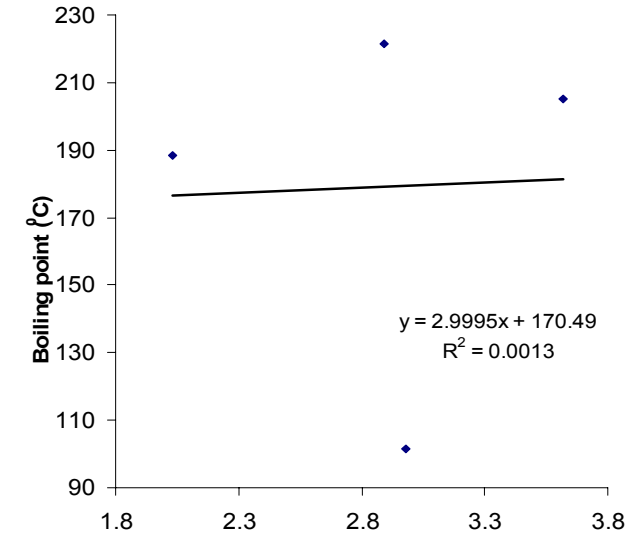

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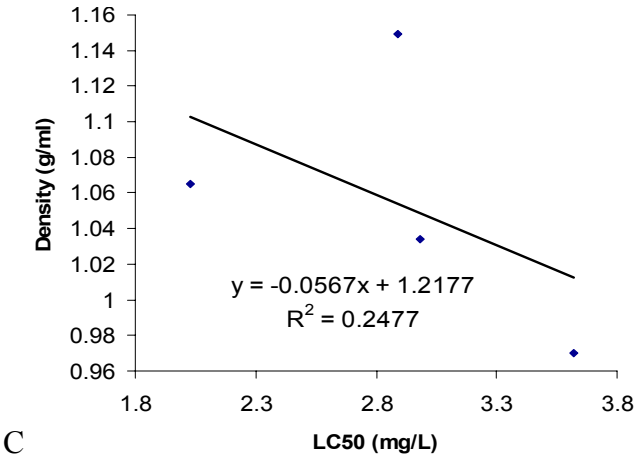
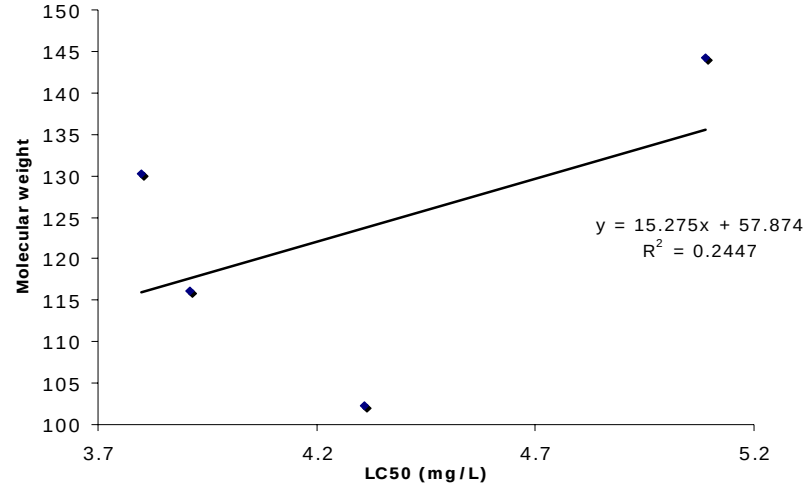


C

Figure 5-4. Regression analyses of the LC_{50} versus the physical properties of each of the 4 heterobicyclics. A) LC_{50} versus molecular weight. B) LC_{50} versus boiling point. C) LC_{50} versus density.



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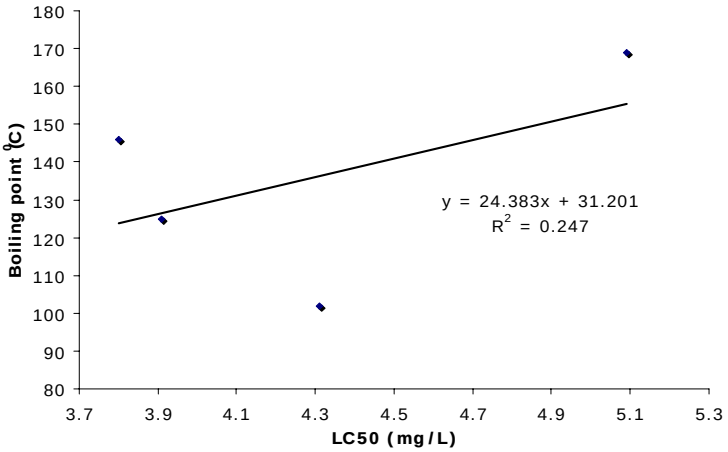

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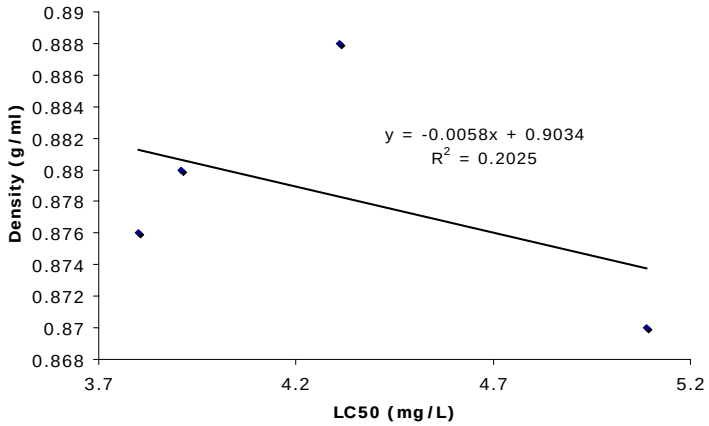
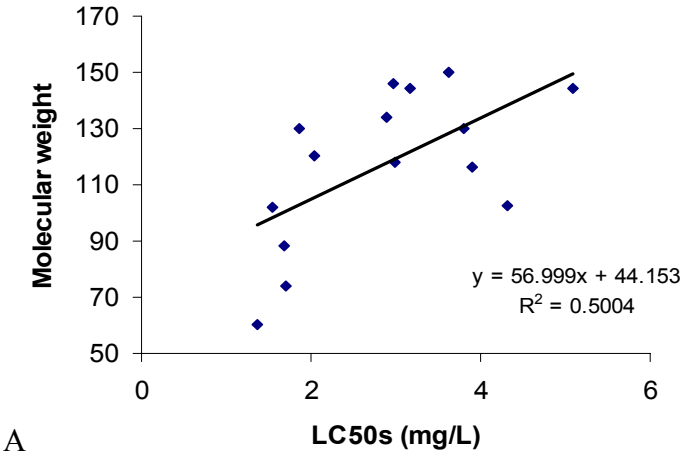


C

Figure 5-5. Regression analyses of the LC_{50} versus the physical properties of each of the 4 acetates. A) LC_{50} versus molecular weight. B) LC_{50} versus boiling point. C) LC_{50} versus density.



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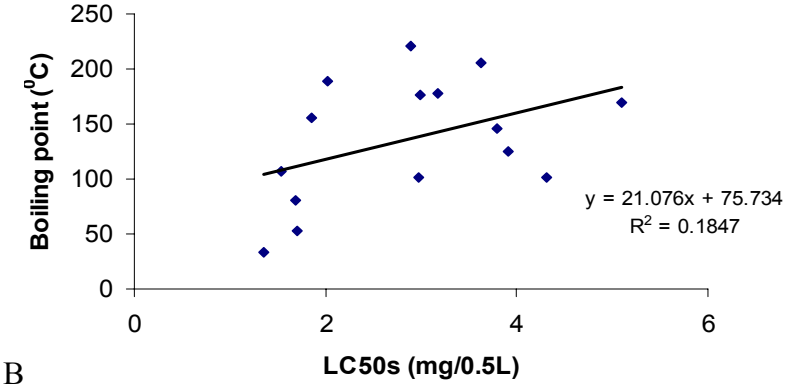

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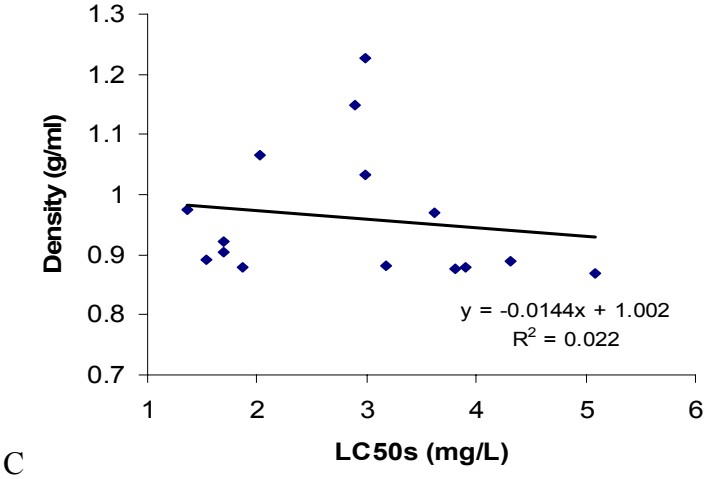

C

Figure 5-6. Regression analyses of the LC_{50} versus the physical properties of all the 15 novel compounds (formates, acetates, and heterobicyclics). A) LC_{50} versus molecular weight. B) LC_{50} versus boiling point. C) LC_{50} versus density.

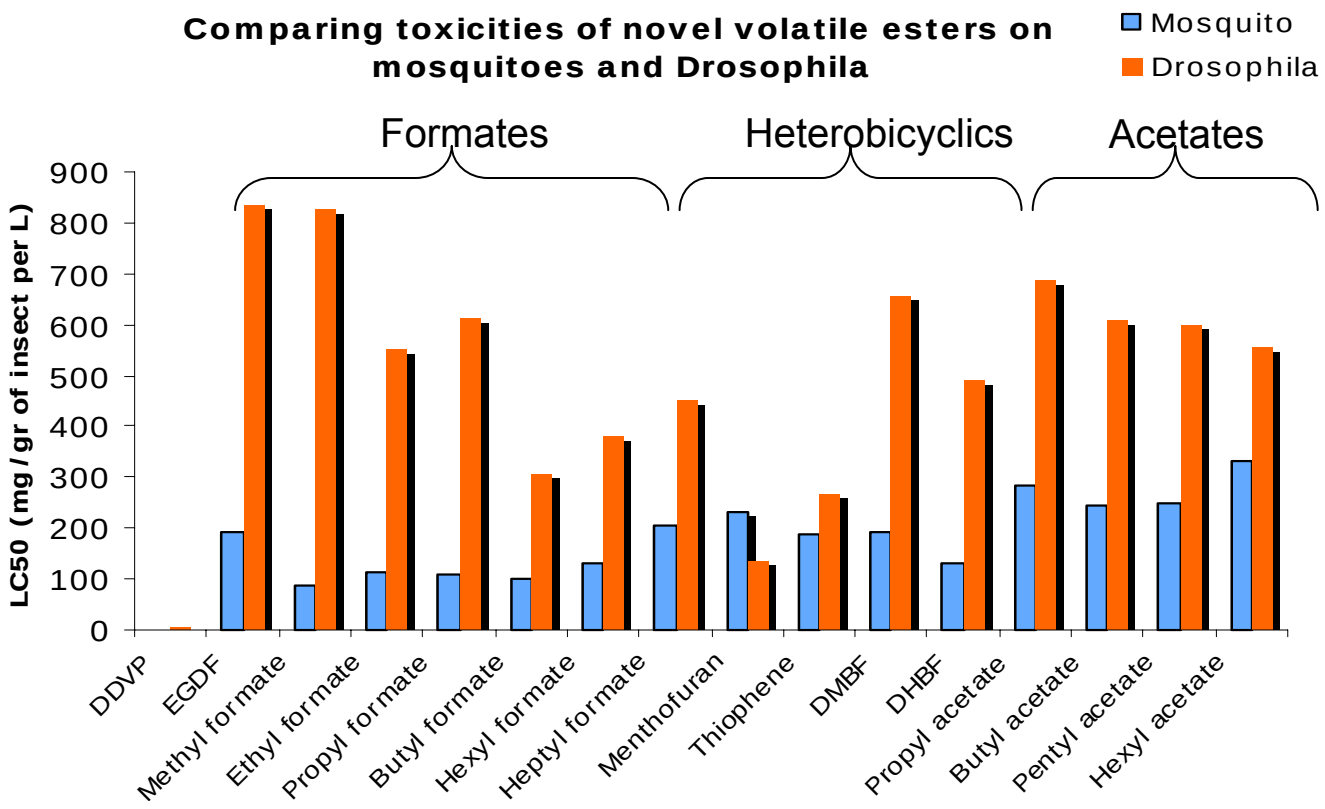

Figure 5-7. Body-weight corrected LC_{50} values for mosquiotes *Aedes aegypti* & *Drosophila* when exposed to the vapors of the 15 low molecular weight esters and the organophosphate DDVP.

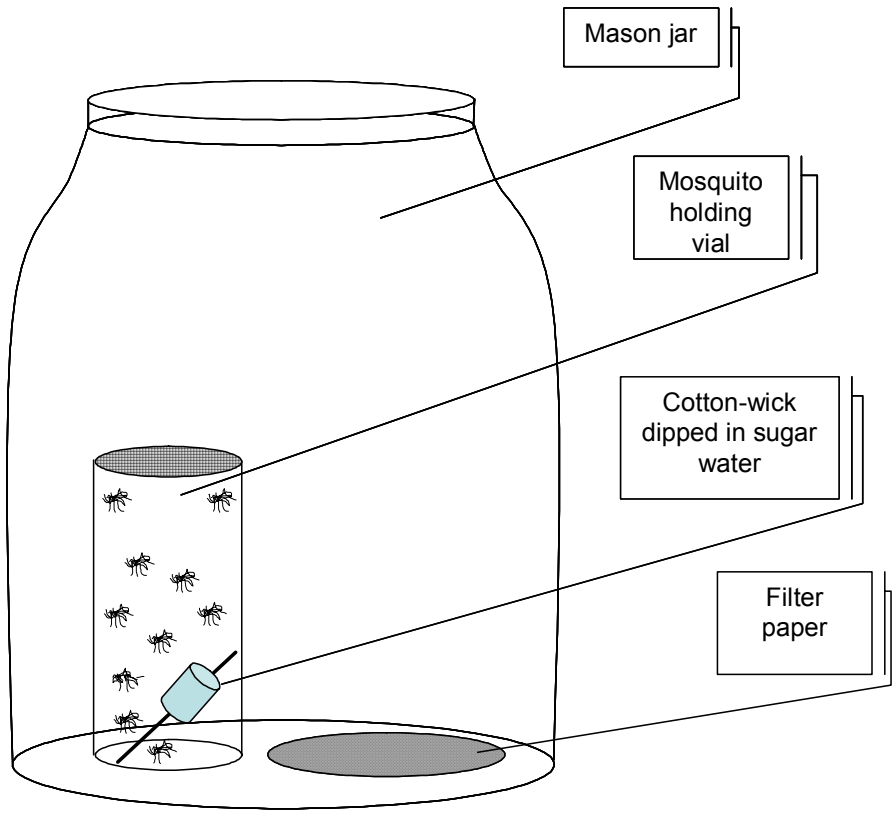

Figure 5-8. Main bioassay set-up.

CHAPTER 6
EVALUATION OF VAPOR TOXICITY OF NOVEL LOW MOLECULAR WEIGHT COMPOUNDS ON HOUSE FLIES

Introduction

Volatile insecticides have been commonly used as fumigants for the control of structural pests and the protection of agricultural properties. However, they have been mostly ignored for the control of medical importance pests such as mosquitoes and flies. Dichlorvos (DDVP) is the one volatile insecticide mostly studied on mosquitoes and flies. Dichlorvos is an organophosphate insecticide and was registered in 1948 (EPA 2006). One very common formulation of dichlorvos is resin strips. Resin strips were initially registered for use in areas where flies, mosquitoes and other nuisance pests occur. Dichlorvos has been classified by the Environmental Protection Agency (EPA) as a "probable human carcinogen", and because of its implications in human health in 2006, its use in homes was restricted to confined spaces such as wardrobes, cupboards and closets (EPA Office 2006). Therefore, there is a need for replacement of dichlorvos with friendlier, less toxic chemistries. Low molecular weight, highly volatile formates, acetates, and heterobicyclics may be potential replacement for dichlorvos.

Thirty novel, low molecular weight compounds with insecticidal activity were tested on *Drosophila melanogaster* Meig. (Scharf et al. 2006). The compounds belonged to six different families: heterobicyclics, formates, acetates, propionates, butyrates and valerates. *Drosophila* was used as a model to assess potential efficacy of these novel chemistries against mosquitoes and flies. Findings showed 7 highly effective compounds with vapor toxicity: four formate esters and three heterobicyclics. The reaction of an organic acid and an alcohol is called esterification, where the end products are always ester and water. Formate esters are organic compounds composed of formic acid and a corresponding alcohol. Acetate esters, similarly to formate esters,

are composed of acetic acid and a corresponding alcohol. On the other hand the structure of heterobicyclics is made from fused 5, 6-membered rings.

For my research I investigated the vapor toxicity effect of three of those compounds, one heterobicyclic (menthofuran) and two formate esters (ethylene glycol diformate, heptyl formate) directly on house flies. Both heptyl formate and menthofuran are naturally occurring compounds. Heptyl formate is naturally found in kumquats and has a floral, apple scent. It is commercially used as a flavoring agent in apple, apricot, kumquat, and wine products to name a few. Menthofuran is naturally found in peppermint oil. It has a musty, nutty odor and is used as a flavoring/odor agent in coffee and chocolate products. In Table 5-1 the chemical structures of the three chemicals can be seen. Information such as molecular weight, boiling point, density, natural occurrence and other physical properties are included in the same table as well.

Materials and Methods

Chemicals

Three novel insecticides (Sigma Aldrich Chemical, Milwaukee, WI) were tested; one heterobicyclic (menthofuran) and 2 formate esters [heptyl-formate and ethylene glycol di-formate (EGDF)]. Dichlorvos (DDVP) was tested as a positive control (Chem Service, West Chester, PA). All insecticides were >99% pure and in a liquid form. Insecticide stock solutions were prepared in acetone at concentrations of 200 or 10 µg/µl. All compounds and stock solutions were held at –20^{o}C in glass vials with rubber lined caps to prevent vapor escape until placed in experiments.

The insecticide synergists SSS-tributyl-phosphorotrithioate (DEF) and piperonyl butoxide (PBO), which are esterase and cytochrome P450 inhibitors respectively, were used (Mobay Chemical Co., Kansas City, MO and MGK Inc., Minneapolis, MN). DEF and PBO were >95% pure. DEF and PBO stock solutions were prepared at 100µg/ml in acetone.

Ceramic Rods

Hydrophilic, ceramic, porous rods (Small Parts, Inc., Miami, FL) were used to provide controlled vapor release of the volatile compound heptyl formate. The rods were 7.5 cm in length and 1.3 cm in diameter. The porous size of the ceramic rods was 2.5 microns and 38% of each rod was void volume. In order to decrease insecticidal release rate, the rods were covered tightly with aluminum foil leaving one end exposed, prior to being treated with insecticide.

Insects

Flies [Horse-Teaching-Unit (HTU) strain of *Musca domestica* (L.)] reared at the University of Florida in Gainesville were used. Flies were reared on a 12:12 (L:D) photoperiod at 26^{o}C and ~55% RH. Fly larvae were fed on a medium containing 3 liters wheat bran, 1.5 liters water, and 250 ml of dairy calf feed (Calf Manna; Manna Pro. Corp., St. Louis, MO) pellets. Fly pupae were separated from the medium and placed into screened rearing cages (40.6 by 26.7 by 26.7 cm) for emergence. Fly adults were maintained on a 2 parts granulated sugar and 1 part powdered milk diet with water *ad libitum*.

Prior to each treatment 3 to 5-d-old adult flies were aspirated from their cages and placed into plastic deli cups on ice until their activity was reduced. Ten females were removed from the deli cups using a feather tip forceps. A minimum of 300 flies were selected for exposure to each insecticide.

Bioassay

Main bioassay set-up. Ten females were transferred from the deli caps into 125 ml plastic vials. Caps with an opening of ~2.6 cm in diameter, covered with common fiberglass window screening (~1.55 mm mesh), were used to close the vials. The screening prevented insect escape while allowing for gas exchange. Along with the house flies a cotton wick (~1.5 cm in length) dipped in 10% w/v solution of sugar water was placed in the vials. A toothpick (~6.3 cm in

length) was used to support the wick. The wick was provided as the nutrient and moisture source. The flies were given 1 h to recover from the chilling effects of the ice prior to the treatment, and then every vial was placed into a Mason 1 liter (1 quart) glass jar along with an untreated filter paper (55mm in diameter). Prior to closing the glass jar the filter paper was treated with the proper quantity of insecticidal solution using an eppendorf pipette. The concentration of the insecticidal solution varied depending on the insecticide tested. Menthofuran was applied at a 200 µg/µl concentration and in a range from 1-4 mg. Pure heptyl formate and pure EGDF were applied in a range from 18-44 mg and 2.5-15 mg, respectively. DDVP was applied at a concentration of 10 µg/µl and in a range from 0.1-0.2 mg. There was also a blank control where the filter paper received no chemical at all, and a solvent control, which received a volume of acetone identical to the highest insecticide solution volume (up to 20 µl). In order to determine mortality the jars were shaken for a minimum of 15 sec before fly movement was observed. A fly was recorded dead when there was no movement observed.

Synergist (DEF and PBO) bioassay set-up. The effect of synergists on the toxicity of the three insecticides tested above was investigated. The synergist bioassay was conducted in the same way described above except that an extra step was added. That extra step involved the exposure of the house flies to the synergist, prior to their exposure on the insecticides. For the synergist bioassay the plastic vials were replaced with 125 ml glass vials to prevent absorption of the synergist into the plastic. Synergist stock solutions at 100 µl were pipetted to every glass vial using an eppendorf pipette, so that every vial would contain 10 µg of the synergist. Previous studies have shown that this synergist quantity causes no insect mortality after 24 h of exposure (Nguyen et al. 2007). After treating the vials with the synergist, the vials were rolled on their sides under a fume hood to ensure equal distribution of the synergist on the inner surfaces while

the acetone evaporated. Once acetone evaporated, 10 house flies were added in every glass vial along with the moist cotton wick and were held for an hour to allow for the synergist to take effect. Along with the blank and the solvent control, a synergist control was added where the flies were only exposed to the synergist.

Controlled vapor release of heptyl formate. Ceramic rods were used to determine effectiveness of heptyl formate in killing house flies over time. For the rod bioassay the house flies were handled the exact same way as described before. Three different treatments were tested and one blank control. In the first treatment flies within the glass jars were exposed to a single rod embedded with 3.81 g of heptyl formate. This treatment was replicated five times. In the second treatment flies were exposed to a filter paper embedded with 0.95 g of heptyl formate, which is the amount of heptyl formate that a single rod is anticipated to release within 24 hrs. In the third and final treatment the insects were exposed to a filter paper embedded with the same amount of heptyl formate as the rods. Mortality was determined every 24 hrs after which the five rods and the treated filter papers were removed to new jars with new insects. The process was repeated over a 9 day period.

Data Analysis

In those cases where control mortality was observed data was adjusted using the Abbott's formula (Abbott 1925). When control mortality exceeded 10% that rep was discarded. Probit analysis was performed and the LC_{50} and LC_{90} of each insecticide with and without the synergist were estimated (SAS Institute 2003). The data reported in Tables 6-1, and 6-2 include slope, goodness of fit characteristics (chi-square, P-value) and LC_{50} and LC_{90} estimates with 95% confidence limits. LC estimates with non overlapping 95% confidence limits were considered significantly different.

Body-weight corrected LC_{50} of each insecticide for house flies and *Drosophila* were calculated. One hundred individuals from both species were weighed and that weight was recorded. That number was then divided by 10 to give the average weight of 10 house flies and 10 *Drosophila* (0.2126 and 0.006 g, respectively). The average weight of the 10 insects was used to adjust the LC_{50} from mg/liter into mg/ g of insect body weight/liter. The *Drosophila* data were retrieved from Scharf et al. (2006).

PoloPlus 2.0 (2005) was used to calculate the potency ratios of the LC50s with & without the synergist. The program calculated 95% confidence limits for every ratio. The 95% CI were used to determine whether there were significant differences in the LC50s due to the effect of the synergists.

In order to determine heptyl formate release rate form each rod regression analysis was performed (SAS Institute 2003) that showed the relationship between release of heptyl formate vapors and time. The rods were weighed before and after being embedded with heptyl formate. The decrease in the rod weight was recorded through time and the release of heptyl formate was estimated. According to the regression equation [$y=0.0006x-0.0003$ and $R^2= 0.9994$, where y represents heptyl formate weight in grams and x represents time of release in minutes] it would require at least 111.11 hours for 4 g of heptyl formate to be released. Also, SNK (Student-Newman-Keuls) test was performed to determine the day when significant decrease in house fly mortality for the rod (3.81 g) treatment was seen (SAS Institute 2003).

Results

Toxicity Evaluation of Novel Compounds

DDVP was by far the most toxic compound tested on house flies. Specifically, it was 25 times more toxic compared to the second best compound, the heterobicyclic menthofuran. Menthofuran was the most toxic compound among the three compounds tested on house flies

(LC_{50} estimate 3.70 mg/liter). EGDF was the second most toxic compound and heptyl formate was the least toxic compound among the three (LC_{50} estimates 9.27 and 32.62 mg/liter, respectively) (Table 6-1, Table 6-2, Fig. 6-1).

Toxicity Evaluation of Novel Compounds with the Synergistic Effect of DEF and PBO

For heptyl formate and EGDF, when co-applied with DEF, their toxicities decreased significantly. The toxicity of heptyl formate decreased by 1.5 times, whereas the toxicity of EGDF decreased by 2 times. Also, the toxicity of menthofuran increased by 1.5 times, when it was synergized with PBO. All synergist effects were significant at the LC_{50} level.

Effectiveness of Controlled Vapor Release of Heptyl Formate in Killing House Flies

The mortality data among the different treatments are shown in Table 6-3. The control treatment caused no mortality throughout the duration of the experiment, which lasted for 9 days. The filter paper treated with 0.95 g of heptyl formate caused 100% mortality for the first day. The filter paper treated with 3.81 g of heptyl formate caused mortality for days 1, 2, and 3. The rod embedded with 3.81 g of heptyl formate caused mortality throughout the duration of the experiment. Also, it was on the 9^{th} day when significant decrease in house fly mortality was seen.

Discussion

Comparing Toxicities of Novel Compounds Among House Flies and *Drosophila*

This study is the first report of the toxic effects of the novel volatile compounds heptyl formate, EGDF, and menthofuran on house flies. Scharf et al. (2006) reported toxicity of these novel compounds on *D. melanogaster* Meig. They used *Drospophila* as a model to assess potential efficacy of these compounds on mosquitoes and flies. There were significant differences among the toxicities of the compounds on house flies and *Drosophila* (Table 6-2). Overall, all the compounds were more toxic to house flies than *Drosophila*. DDVP was by far the most toxic insecticide for both insects and was significantly more toxic to house flies than

Drosophila. Specifically, DDVP was 5.2 times more toxic to *Drosophila* than house flies. DDVP has been known as a very effective insecticide against various insects for many years. Ihnidris and Sullivan (1956) gave one of the earliest reports regarding the toxicity of DDVP against house flies. They reported 100 % knock down of house flies after 2 hours exposure to DDVP vapors. On average the compounds were approximately by 10 times more toxic to house flies than *Drosophila*. Menthofuran was the most toxic compound when tested on both insects. However, heptyl formate was more toxic than EGDF to *Drosophila* and less toxic than EGDF to house flies.

There are several possible explanations as to why the compounds performed differently on house flies and Drosophila. A first explanation could be differences on the insect handling techniques during the experimentation. Scharf et al. (2006) used CO_2 to knock down *Drosophila* prior to exposing them on the insecticides, whereas I used ice for knocking down house flies. Another explanation could be species differences on acquiring and metabolizing insecticides. The lethal effects of insecticidal compounds depend upon the amount of insecticide that reaches the target site (site of action). The amount of insecticide that reaches the target site is controlled by certain processes such as penetration through the insect cuticle, diffusion through the insect spiracles, bioactivation/biodegradation within the insect body, travel distance to the site of action and finally excretion just to name a few (Quraishi 1977, Matsumura 1980, Yu 2006). Different insect species can differ greatly in susceptibility to insecticidal compounds due to distinct differences in the physical and physiological processes mentioned above. Insecticides with high vapor pressures, such as the insecticides studied in this paper, show the tendency to enter the insect body through the spiracles (Matsumura 1980). Therefore, the susceptibility of an insect to a vapor toxicant is believed to be correlated with its rate of respiration (Vincent & Lindgren

1965). In general, different insects exhibit different patterns of gas exchange when at rest (Lighton 1988, 1990, Lighton & Berrigan 1995). The most familiar and well studied pattern is the Discontinuous Gas Exchange Cycle (DGC) (Kestler 1985, Lighton 1994), where the spiracles remain close for lengthy periods of time allowing for no gas exchange. This close-spiracle phase is followed by a fluttering-spiracle phase and finally an open-spiracle phase where the accumulated CO_2 escapes form the tracheal system to the surrounding environment. Little information, however, is available on the respiratory pattern of small insects (body weight ~ 1 mg) such as *Drosophila* (Williams et al.1997, Williams and Bradley 1998, Lehman et al. 2000, Fielden et al. 2001). What is known so far is that *Drosophila* has the ability to control gas release from its tracheal system. There is some evidence to support that *Drosophila* performs DGC, however further research is needed for more legitimate results. Not much research has been done on the respiratory pattern of houseflies. Due to the absence of evidence one should consider that house flies and *Drosophila* may follow a different breathing pattern. If this is true, the insects may be allowing different amounts of insecticide to enter their body and that may be one of the factors responsible for the different responses they show to the various insecticides tested.

Another explanation could be differences in the detoxification systems among house flies and *Drosophila*. Once the insecticide enters the insect body it is perceived as a foreign substance or xenobiotic, and is metabolized by different metabolical processes with the ultimate goal to be converted into a less toxic polar substance that will eventually be removed form the body. This metabolic process is called “detoxification”. By far the two most significant reactions involving the metabolism of insecticides are the NADPH-requiring general oxidation system and the hydrolysis of esters (Matsumura 1980). The activity of these two enzymatic systems varies

among different insect species, resulting in species differences in susceptibility to various insecticidal compounds (Casida et al. 1976. Brooks 1986, Valles et al. 1997, Scharf et al. 2000).

Implications of the Synergistic Effects of PBO and DEF on the Toxicity of the Novel Compounds on House Flies

The modes of action of menthofuran, EGDF, and heptyl formate on house flies so far remain undefined. According to the work presented in this paper there seems to be a significant effect of cytochrome P450 enzymes on the metabolism of menthofuran. When cytochrome P450 enzymes were inhibited by the action of PBO the toxicity of menthofuran increased by 1.5 times. This finding is in agreement with Nguyen et al. (2007), who showed that P450 plays an important role in methofuran detoxification. They, also, showed evidence supporting P450-based activation of menthofuran.

Also, there was evidence supporting esterase-based activation of both heptyl formate and EGDF. When heptyl formate and EGDF were co-applied with the esterase inhibitor DEF their toxicity decreased by 1.5 and 2 times, respectively. The second finding comes in agreement with both Haritos & Dojchinov (2003) and Nguyen et al. (2007), who supported esterase-based activation of some formate esters. In order for more legitimate conclusions to be made more extensive and complete research needs to be conducted where all of the compounds will be tested in combination with both synergists on susceptible and even resistant fly species.

Structure-activity Relationships

Among the two formate esters EGDF and heptyl formate, the first is significantly more toxic to houseflies than the second by 3.5 times. When looking at their chemical structures there is one difference that stands out; EGDF is composed by two molecules of formic acid, where as heptyl formate is composed by only one. According to Haritos & Dojchinov (2003) findings on alkyl ester mode of action, formate esters were more toxic than other alkyl esters. That was

partially due to their hydrolysis to formic acid. Based on their findings one might say that EGDF was more toxic than heptyl formate because it contains two molecules of formic acid in its structure, and therefore the esterases would release 2 molecules of formic acid when hydrolysing EGDF, as opposed to 1 molecule of formic acid when hydrolyzing heptyl formate.

Controlled Vapor Release of Heptyl Formate

Controlled vapor release of heptyl formate can provide effective house fly mortality over time. In the future these compounds should be embedded in specialized plastic polymers, similar to the DDVP resin strips, that would provide prolonged release of vapors and, therefore, result in prolonged insect mortality.

In conclusion, DDVP was by far the most toxic insecticide for both insects and was significantly more toxic to house flies than *Drosophila*. All three novel compounds were significantly more toxic to house flies than *Drosophila*. Menthofuran was the most toxic compound among the three tested on house flies (LC_{50} estimate 3.70 mg/liter). EGDF was the second most toxic compound and heptyl formate was the least toxic compound.

Table 6-1. Vapor toxicity of EGDF, heptyl formate, and menthofuran with and without the synergistic effect of DEF and PBO and the organophosphate DDVP to house flies *Musca domestica* (L.)

Insecticide	Slope ± SE	LC_{50} mg/liter (95%CI)	LC_{90} mg/liter (95%CI)	χ^2	P	Potency Ratio [b]
DDVP[a]	9.6 ± 1	0.148 (0.14-0.15)	0.202 (0.19-0.22)	2.35	0.67	-
EGDF	7.4 ± 0.8	9.27 (8.75-9.75)	13.81 (12.81-15.36)	9.76	0.14	
EGDF + DEF	7.9 ± 0.67	18.56 (17.76-19.33)	26.88 (25.34-29.02)	8.10	0.23	2 (1.87-2.14)
Heptyl formate	4.1 ± 0.6	32.62 (30.21-35.44)	66.89 (55.88-91.58)	3.87	0.79	
Heptyl formate + DEF	6.9 ± 1.19	48.70 (46.20-51.63)	74.45 (65.94-94.29)	3.76	0.29	1.5 (1.36-1.64)
Menthofuran	10.6 ± 1.13	3.70 (3.58-3.83)	4.88 (4.61-5.32)	4.29	0.12	
Menthofuran + PBO	4.8 ± 1.22	2.43 (1.85-2.69)	4.49 (3.90-6.59)	0.49	0.48	0.65 (0.56-0.76)

[a] Positive control
[b] LC_{50}+synergist / LC_{50}

Table 6-2. Body-weight corrected vapor toxicities of EGDF, heptyl formate, menthofuran and the organophosphate DDVP to house flies *Musca domestica* (L.) and *Drosophila melanogaster* Meig.

Treatment	Housefly LC_{50} (mg/g of insect/liter)	Drosophila LC_{50} (mg/g of insect/liter)[b]
DDVP[a]	0.7 (0.67-0.72)	3.7 (3.2-4.3)
EGDF	44 (41.15-45.86)	834 (676.6-1,846)
Heptyl formate	153 (142.1-166.7)	450 (420-475)
Menthofuran	17.4 (16.83-18)	136 (120-150)

[a] Positive control
[b] *Drosophila* data Scharf et al. 2006

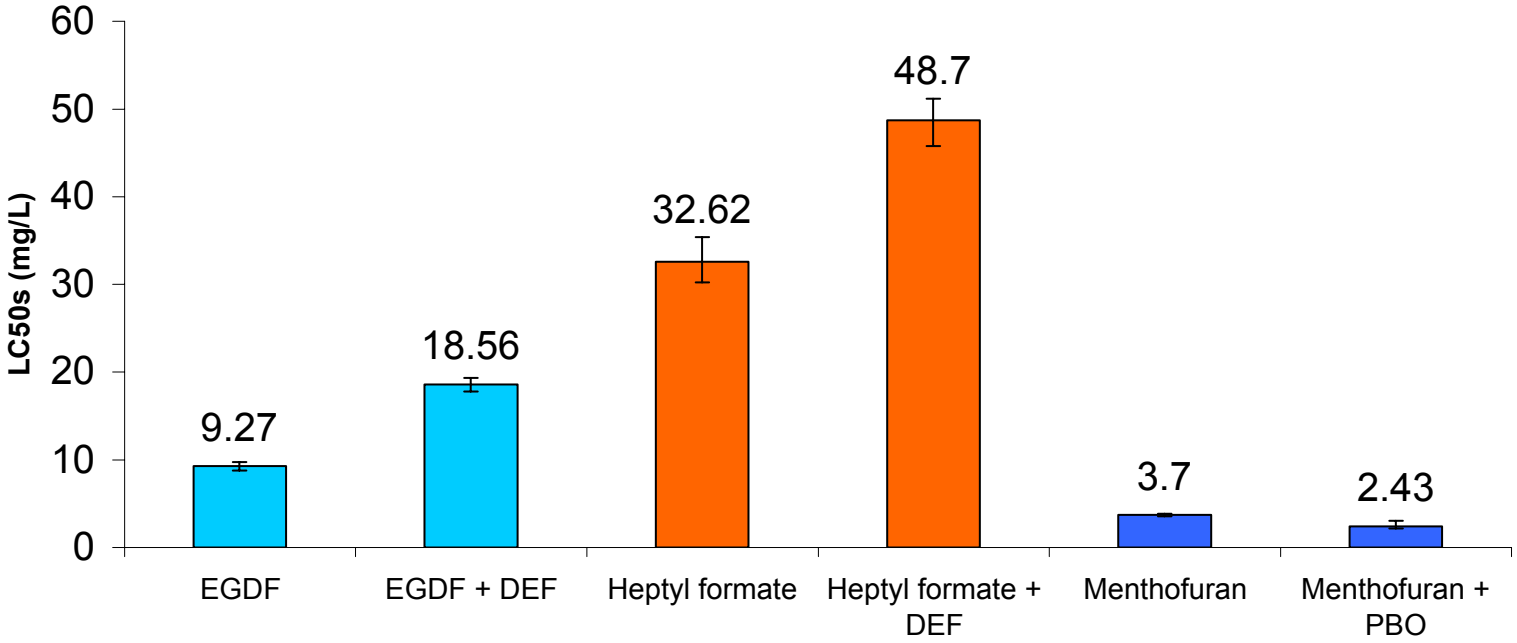

Figure 6-1. Vapor toxicity of EGDF, heptyl formate, and menthofuran with and without the synergistic effect of DEF and PBO to the house flies *Musca domestica* (L.).

Table 6-3. Percent mortality of controlled vapor release of heptyl formate on house flies Musca domestica (L.) over 9 days among 3 different treatments and a blank control

Time (days)	Percent Mortality of Heptyl Formate on House Flies			
	Control	Filter Paper (3.81g)	Filter Paper (0.95 g)	Ceramic Rod (3.81 g)
Day 1	0	100	100	100a
Day 2	0	100	0	100a
Day 3	0	100	0	98 ± 2a
Day 4	0	0	0	96 ± 4a
Day 5	0	0	0	88 ± 5.8ab
Day 6	0	0	0	84 ± 6.8ab
Day 7	0	0	0	82 ± 5.8ab
Day 8	0	0	0	74 ± 10.3ab
Day 9	0	0	0	64 ± 14.7b

Percentages followed by the same letter are not significantly different (SNK test, SAS Institute, 2003)

CHAPTER 7
SUMMARY

The main objective of my research was to evaluate vapor toxicity of novel, low molecular weight compounds with insecticidal activities on mosquitoes and house flies. The results of the experiments have shown that all compounds demonstrated vapor toxicity to both mosquitoes and house flies. However, the organophosphate DDVP was by far the most toxic compound to both mosquitoes and house flies.

A total of 16 insecticidal compounds were tested on mosquitoes: 15 novel compounds (7 formates, 4 acetates, and 4 heterobicyclics) and the organophosphate DDVP. DDVP was 54.4 times more toxic compared to the second best compound, the formate ester methyl formate. Within the novel compounds, overall, formate esters were the most toxic family followed by the heterobicyclics and, last, by the acetate esters. Within the formate group, methyl formate was the most toxic ester (LC_{50} estimate 1.36 mg/liter), followed by butyl, propyl, ethyl, hexyl formate, EGDF, and heptyl formate. The toxicities of propyl and ethyl formate were not significantly different and there was no major significance between them and butyl formate. EGDF and heptyl formate (LC_{50} estimates 2.99 and 3.17 mg/liter, respectively) were the least toxic formate esters with toxicities in the same range as the heterobicyclics, the second best performing family of compounds. Within the heterobicyclic group, coumaran was most toxic (LC_{50} estimate 2.03 mg/liter), followed by benzothiophene, dimethyl-coumarone and menthofuran. Benzothiophene and dimethyl-coumarone were not significantly different. Within the acetate group, hexyl acetate was the least toxic compound (LC_{50} estimate 5.09 mg/liter). The toxicities of propyl, butyl and pentyl acetate were not significantly different.

A total of 4 insecticidal compounds were tested on house flies: two formates, one heterobicyclic, and the organophosphate DDVP. DDVP was 25 times more toxic compared to

the second best compound, the heterobicyclic menthofuran (LC_{50} estimates 3.70). Menthofuran was followed by the formate esters EGDF and heptyl formate (LC_{50} estimates 9.27 and 32.62 mg/liter, respectively).

DDVP has been characterized by EPA as a "probable human carcinogen" and because of its implications in human health its use in 2006 was restricted to confined spaces such as wardrobes, cupboards and closets where no human activity takes place (EPA 2006). Even though the novel compounds did not demonstrate the high vapor toxicity demonstrated by DDVP, they showed good potential to be used as alternative vapor toxicants against mosquitoes and house flies for those situations where the use of DDVP is banned. Their low mammalian toxicities in combination with their pleasant, fruity odors make them very good DDVP replacement candidates. Also, the potential of the novel compounds as contact toxicants should be investigated in the future as they might exhibit good toxicities as contact insecticides, and thus provide an additional tool for the control of public health pests such as mosquitoes and house flies.

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BIOGRAPHICAL SKETCH

Alexandra Chaskopoulou was born on June 3, 1981 in Thessaloniki, Greece, to Efthimios and Kalliopi Chaskopoulos. She has one sister and one brother. She and her family have spent most of their lives in Greece. Upon completion of her high school education in Greece, she decided to come to the United States in order to pursue her college education as an entomologist.

She arrived at the United States in 2003, and within a year she earned her minor in biology from St. Andrews University of Michigan. In 2004 she moved in Gainesville, Florida where she earned her Bachelor of Science degree in entomology from the University of Florida and graduated in 2005. She remained at the University of Florida since 2007, when she completed her master's degree in medical and veterinary entomology.

POTENTIAL OF INSECTICIDE-TREATED CORDS AND SPRAYABLE BAITS FOR CONTROL OF HOUSE FLIES (DIPTERA: MUSCIDAE)

By

JEFFREY CONRAD HERTZ

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

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To my wonderful family

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POTENTIAL OF INSECTICIDE-TREATED CORDS AND SPRAYABLE BAITS FOR CONTROL OF HOUSE FLIES (DIPTERA: MUSCIDAE)

By

Jeffrey Conrad Hertz

August 2007

Chair: P.G. Koehler
Major: Entomology and Nematology

House flies are often controlled using insecticides when the source of infestation can not be located or remedied by non-chemical methods. Historically, house flies have shown a tremendous potential to develop insecticide resistance and with few classes of insecticides currently registered for house fly control, new products and methods need to be evaluated to prevent future control failures. This research evaluated the potential use of two innovative methods to control house flies: fipronil- and indoxacarb-impregnated cords and a sprayable imidacloprid fly bait.

For the insecticide-impregnated cord studies, eight various natural and synthetic cords were evaluated to determine which cords were attractive to house flies. Natural cords were more attractive than synthetic cords; the plant-based manila cord was most attractive and the nylon parachute cord was least attractive. The most attractive cords (manila, cotton, wool, nylon, and polypropylene) were treated with 0.1% fipronil or 0.6% indoxacarb and evaluated in the laboratory to determine their effectiveness. All cords were more effective than the impregnated cotton cord except the fipronil-impregnated nylon cord (LT_{90}) and the indoxacarb-impregnated polypropylene cord. The wool cord was the most effective, LT_{50} (Fipronil = 12.9 h; Indoxacarb = 32.6 h) and LT_{90} (Fipronil = 22.4 h; Indoxacarb = 51.5 h). The wool cords were impregnated

with 0.1% fipronil and 1.2% indoxacarb and evaluated in a controlled field environment with fresh cords and cords that were aged 4 wk. No significant differences were seen between fly count reductions of either treatment. Both treatments reduced fly counts by >57% by 24 h and >87% by 48 h with both fresh and aged cords. A reduction in efficacy was seen with aged cords.

The new imidacloprid sprayable fly bait formulation was compared against two commonly used dry scatter baits in the laboratory and against a granular imidacloprid paint-on bait in a controlled field setting. Additionally, the sprayable bait was evaluated for use in impregnated cords. No differences were seen in mortality between the three scatter baits in the laboratory or between the imidacloprid baits in the field cages. Both imidacloprid baits reduced fly counts by >70% in the field within 24 h, but were not effective after treatments were aged for 2 wk. When various cords were treated with 2.5% of the new bait, the wool cord had higher mortality (74%) compared to the other natural and synthetic cords tested. Knockdown recovery was observed with all bait-treated cords in the laboratory, but was not determined to occur in the field cages. The bait-treated cords reduced fly counts by >82% with fresh cords and cords aged for 4 wk.

Impregnated cords and the new sprayable bait should prove to be valuable tools in established fly management programs in urban, agriculture, and military settings. Fipronil and indoxacarb are not currently registered for house flies, but both appear to be effective insecticides for their control.

CHAPTER 1
STATEMENT OF PURPOSE

Throughout history flies have undoubtedly been a nuisance to both man and animal alike; however, because of their propensity to frequent pathogen-rich filth they do pose a human health risk. House flies have been shown to transmit numerous pathogens and their synanthropic behavior may make house flies one of the most troublesome insect vectors (West 1951, Greenberg 1973). This is especially true in areas affected by natural disasters or conflict. Often times following these chaotic events, basic sanitation measures are out prioritized for casualty recovery and, as a result, tremendous populations of house flies emerge.

Chemical insecticides are often used in these situations or any situation where rapid house fly control is needed. Today there are more chemicals registered as insecticides than ever before, but few of these insecticides are registered for house fly control. The insecticides that are registered for house fly control only come from five chemical classes: organophosphates, carbamates, pyrethrins/-oids, triazines, and neonicotinoids. The organophosphates and the carbamate insecticides continually get further restrictions by the Environmental Protection Agency (EPA) limiting their use and their future availability in the United States may be bleak. In addition, house flies have consistently shown the ability to develop resistance to all chemicals used to kill them (Liu and Yue 2000, Scott et al. 2000, Kaufman et al. 2001) and having only 3-5 chemical classes to rotate with may prove to be detrimental to a fly management program. There is an immediate need for new insecticides registered and new techniques for house fly control to prevent future control failures.

In 2004, the Department of Defense (DOD) established the Deployed War-Fighter Protection (DWFP) program to develop and test management tools for pest and vector species, including house flies, which transmit diseases to the deployed war-fighters. The Armed Forces

Pest Management Board (AFPMB) administers the DWFP program and specifically requested research to improve or develop integrated filth fly control strategies and non-conventional pesticide methodologies. Insecticide-impregnated cords and sprayable fly bait may be beneficial tools that the deployed war fighter could use for fly management programs. The research contained herein was designed to provide new information regarding these techniques to the DOD.

CHAPTER 2
REVIEW OF LITERATURE

Classification, Origin, and Distribution

The house fly, *Musca domestica*, belongs in the class Hexapoda, order Diptera, suborder Brachycera, infraorder Muscomorpha (Cyclorrhapha), and family Muscidae (Triplehorn and Johnson 2005). It was first described by Linnaeus (1758).

It is believed the family Muscidae evolved sometime during the Permian period of the Paleozoic era (Lambrecht 1980). The exact origin may never be known, but many speculate that the house fly originated in the Middle East area of the Palearctic region (Skidmore 1985, Pont 1991) and was distributed through multiple introductions into the New World (Marquez and Krafsur 2002). Today, house flies are one of the most commonly found synanthropic pests. It is found in virtually every region of the globe that man or animal exist. The only exception is areas, such as high altitudes and the arctics, which are prone to extreme cold temperatures (West 1951).

Identification

Egg

House fly eggs are white, bluntly rounded, banana-shaped eggs approximately 1 mm in length, and often laid in clusters (Keiding 1976). The egg widens in size posteriorly to anteriorly and the dorsal surface has two longitudinal, curved ridges that narrow just prior to reaching the caudal end.

Larva (maggot)

House flies have three larval instars. Each instar has no eyes, legs, antennae, or appendages and are commonly known as maggots (Moon 2002). The maggot has a rounded posterior that tapers to a point towards its head. A pair of black spiracular plates is located

posteriorly, which progressively becomes more chitinized and "D-shaped" through molts. First and second stage larvae have two spiracular openings (slits) used for gas exchange and a third opening appears on the third instar larvae (Moon 2002). Prothoracic spiracles are fan-shaped and appear after the first molt. A cephalopharyngeal skeleton, comprised mainly of sclerotized "mouth" hooks, is located at the anterior end of the larvae.

Pupa

House fly pupae are approximately 6.3 mm in length (West 1951). At the beginning of pupation, the puparium is white in color but eventually becomes reddish-brown. The puparium is medially enlarged with bluntly rounded ends. Two pupal horns are located laterally just prior to the posterior boundary of the first abdominal segment (Siriwattanarungsee et al. 2005). Posterior spiracles are located on the posterior end and appear as two flat, circular prominences. The anterior spiracle is situated on the puparium in the same location as in the third instar larvae (Siriwattanarungsee et al. 2005).

Adult

The adult house fly is a medium-sized (6-9 mm) gray insect with large brown compound eyes (Moon 2002). On the vertex, between the eyes, lies the ocellar triangle containing the three simple eyes. The house fly's antennae are also located between the eyes, within the triangular facial depression. The antenna is six segmented, but only appears to be four. The first three segments, the scape, pedicel, and large first flagellar segment, give rise to the three-segmented arista. Segment one and two of the arista is ambiguous; segment three is bristlelike. The sponging proboscis of the house fly terminates to a heart-shaped sucker. The proboscis can be greater than the length of the head when fully extended or obscure when fully retracted. Two brownish-black maxillary palpi lie on the anterior margin of the proboscis.

The thorax of the house fly has four black longitudinal stripes that can be viewed dorsally. Attached laterally to the mesothorax are two membranous wings. The wings, when extended, are approximately twice the distance of the fly's length. At rest, the house fly pulls the wings back incompletely over the abdomen forming an overall triangular appearance from above. The fourth longitudinal wing vein sharply angles towards the wing apex. Situated below each wing is a knob-shaped organ used for equilibrium called the haltere. The legs of the house fly attach ventrally to each segment of the thorax and all legs have five-segmented tarsi. The first tarsal segment is much longer than all other segments and the fifth segment bears two claws, a hair-like empodium, and a sticky pad called a pulvillus.

The abdomen is gray, dorsally, and cream-colored ventrally. Five pairs of spiracles line the ventral surface of the female; six pairs line the ventral surface of the male. The tip of the abdomen ends in either the sclerotized genitalia of the male or the retracted ovipositor of the female.

Sex Differentiation

Adult female house flies are almost always larger than adult males. Additionally, males can be differentiated from adult females by locating the dark sclerotized genitalia plate located on the distal aspect of the abdomen. The tiny mark made by the ovipositor tip of the female is very distinctive compared to the male genitalia especially when females are gravid. Furthermore, adult house flies can be separated by the gap distance that divides the compound eyes. Females have a much wider space separating the eyes when compared to male counterparts. No differentiation can be made in the immature stages.

Life Cycle

Male and female house flies can successfully mate 24 hours after emergence from the pupae (Murvosh et al. 1964). Prior to copulation, a male will seize a resting female or *strike* a

flying female at which point they fall to a surface. If a copulating pair is disturbed while mating they may attempt to fly a short distance to an alternate surface. Copulation can last for more than 1 h, but sufficient sperm transfer can occur in less than 10 min (Murvosh et al. 1964). Once successful copulation takes place, the female is fertilized for life. Batches of up to 150 eggs are laid 4-8 days after copulation (West 1951). The female house fly carefully embeds her eggs into practically any fermenting organic material. The eggs hatch within 24 hours, and 1st instar larvae emerge and begin to feed (West 1951). The larvae undergo two molts within 3-5 days before pupation (Hogsette 1995). Pupation begins when the 3rd instar larva stops feeding and constricts within its own integument. This makes a white puparium which turns reddish-brown within 24 hours. After 3-5 days, the adult breaks through the anterior end of the puparium using a temporary, inflated sac located on its head called the ptilinum. Once free from the puparium, the newly emerged adult house fly hops around to let its wings extend and cuticle harden.

Nutrition, Longevity, and Overwintering

House flies larvae have been reared in the laboratory on practically every type of filth imaginable. Today, they are most often reared in a medium containing animal feed and water (Hogsette 1992). Fermenting odors attract gravid females to oviposit on breeding sites in the field, but understanding precisely what nourishes a maggot within the medium is not fully understood. All house fly maggots are saprophagous and feed on liquids or substrates that are readily dissolved by droplet regurgitation (Nation 2002). It was originally thought that bacteria were essential in the development of house fly maggots, however many have successfully reared them in aseptic media (Brookes 1956, Monroe 1962). Despite this, bacteria still may have provide nutritional value (e.g. vitamins) to maggots (Zurek et al. 2000).

Adult house flies are omnivorious and emerge with little stored energy and nutrients (Moon 2002). They begin to feed within 2-24 hours after emergence (Keiding 1976). In order to

survive, they must find a sugar source, or other assimilable starch, and water (West 1951). In addition, female house flies require a protein source for vitellogenesis.

When feeding, adult house flies are attracted first visually, then when they are within a detectable range, by smell using their antennae (Keiding 1976). Flies locate the source of the aroma by smelling the substrate with chemoreceptors located on the lateral aspect of their 2-5 tarsi. Once their tarsi are in contact with a suitable substance, the fly extends its proboscis and begins to feed. Liquid substances can be readily imbibed, but solids are ground down using the prestomal teeth on the proboscis and emulsified using a vomit drop originating in the crop and salivary glands. The largest particle a house fly can ingest is 40 μ (Greenberg 1973). Ingested liquid and emulsified particles enter the pseudotracheae and then pharynx. Once past the pharynx, liquids pass into the crop and emulsified food particles enter the proventriculus then the ventriculus. The crop is connected to the pharynx by a long slender tube lined with numerous sphincters that controls the flow of liquid back to the abdomen where the bilobed crop is housed. The heamolymph osmotic level dictates the rate the crop empties into the ventriculus. The more concentrated the sugar meal, the slower the crop empties (Greenberg 1973). The ventriculus empties into the longest part of the alimentary track, the proximal intestine. The proximal intestine is divided from the distal intestine by excretory organs called the Malpighian tubules. The distal intestine terminates at the anus.

Longevity of any organism can be highly variable. Food availability, environmental conditions, and activity of an individual fly greatly influences how long it will live. Of the three survival-mandated nutrients, sugar is the most critical for survival. Lysyk found that the availability of sucrose was the most important factor promoting longevity, followed by other food sources (manure, milk) and temperature (1991). Flies will live 50% longer on sucrose

alone, than they do on water alone (Greenberg 1960). However, flies without water generally die within 48 hours (West 1951).

Temperature is inversely proportional to the life span of the adult house fly; higher temperatures reduce the life expectancy, while lower temperatures increase it (West 1951). In laboratory conditions where adequate food is provided *ad libitum* and environmental conditions are controlled, male house flies can live up to 40 days and female house flies can live up to 60 days (Rockstein 1957). In the field, house flies are estimated to only live about 10 days (Hogsette 1995). Bucan and Sohal (1981) found that adult males and females isolated from the opposite sex live longer than when they are housed together.

To increase their survivability when temperatures drop below optimum levels, house flies survive by overwintering in buildings and animal confinements. All life stages are susceptible to subzero temperatures, so microclimates must exist that allow flies to propagate. Rosales et al. (1994) concluded that house flies require habitats that are above -5ºC, and must stay above 10ºC long enough for the house fly to complete its life cycle.

Flight, Movement, and Resting Behavior

Flies have two wings located on the lateral aspect of the body on the pteropleura. Directly above the metathoracic coxae are the vestigial wings, or halteres, which are used as gyroscopes for equilibrium. Like other flying insects, a house fly achieves flight by creating wing movement through indirect thorax compression and decompression caused by the flight muscles. These flight muscles comprise approximately 11% of the total body weight in the genus *Musca* (Greenberg 1973). This musculature makes house flies extremely strong fliers and very capable of flying upwind in mild and moderate winds.

Fly movement can be classified as dispersal, dispersion, or migration (Greenberg 1973). Dispersal is any active movement within a relatively small defined area. House fly problems are

often localized near a source of infestation (Howard 2001, Nazni et al. 2005). Often times, dispersal will be dependant on the sun light. Flies tend to follow the sun and more will be located where the sun is shining (Anderson 1964). This is especially true in cooler temperatures; in hot temperatures, flies may avoid the sun and search for cooler locations. Dispersion is the movement of flies between adjacent areas and often involves the movement assisted by passive transport. Passive transport can occur on garbage trucks, tractors, automobiles, or any other vehicle including strong winds. This is often seen when breeding sources are sporadic or when no breeding sources are near and flies move into the area in search of new oviposition sites. This movement is why flies are found in areas where no apparent fly breeding material is present. Migration is any directed and sustained flight that often occurs seasonally. This type of movement is not normally associated with house flies, however, many have been trapped in areas that would suggest that migration was the only possible explanation (West 1951, Jones et al. 1999). Passive transport may also play a large role in these situations.

Fly movement can be influenced by many factors such as odors, wind, weather, time of day, and population structure. Food and oviposition sites are probably the most critical factors (Bishopp and Laake 1921). However, many questions still need to be answered on what is attractive or needed by house flies since flies will often leave one oviposition site in search for an alternative site although sources are immediately available and sufficient for survival. When seeking these new food sources or oviposition sites, flies can fly upwind in mild to moderate wind speeds, but strong winds, or even shifting winds, can disperse house flies to areas where survival may not be suitable. Taylor (1974) found that house flies are day fliers and their flight activity increases with high temperatures (25-30ºC) and low humidity (50-65%). Flies always

seek temperatures above 15.5ºC, but are capable of flight at temperatures below 55ºC (Greenberg 1973).

House flies have distinct resting behavior. In warmer climates, flies prefer to rest at night outdoors on low hanging twigs of trees and bushes, but may still be seen resting indoors on wires or cords close to the ceiling (Scudder 1949, Keiding 1965). In colder climates, house flies will rest exclusively indoors. In all cases, flies tend to rest on objects with distinct edges less than 4.5 m from the ground, shielded from direct wind (Scudder 1949). Keiding and Hannine (1964) found a distinct preference for house flies to rest on objects suspended vertically from ceilings, however, Fay and Lindquist (1954) found no differences in orientation of suspended cords.

The visual orientation of house flies to objects has been widely disputed. Objects that are light in color, smooth, or metallic are highly avoided by flies; whereas, objects that are dark in color and rough are generally more frequently rested upon by flies (Arevad 1964). Hecht et al. (1968) performed a number of indoor and outdoor experiments to determine the attraction of house flies to different colored cardboards. They found that black was most preferred indoor and white was the most preferred outdoor. When combining the indoor and outdoor results, the red colored surfaces were most preferred. The least preferred colors were blue (indoor) and brown (outdoor). The attraction to the white surface outdoors was attributed to a fly's attraction to ultraviolet light because of the reflective qualities of the white cardboard. Flies see wavelengths between 350-480 mμ (McCann and Arnett 1972). Contrasts of colors (dark on light/light on dark) may be very important to the attraction of house flies. Howard and Wall (1998) counted more flies on white surfaces with black backgrounds than on any other black/white combination.

Pest Status and Health Importance

House flies are renowned for their ability to annoy anyone and anything they are near. It only takes one house fly to turn a customer away from a restaurant and only a few to disrupt

production and morale at a work site. Most see house flies as a sign of unhygienic conditions and attempt to avoid them at all costs. Flies leave fecal and vomit spots on work equipment, consumables, and personal items most of which are not be generally quantified in economic losses but their impact can easily be seen when comparing two similar establishments one with a fly problem and the other lacking a problem. Litigation cases due to house flies have increased in the United States recently due to the migration of urban dwellers deeper into rural settings where livestock and poultry farms have a relatively large abundance of flies – a situation many urban dwellers may not be familiar with.

Although primarily nuisance pests, house flies do pose a risk to the health and well-being of man and livestock. Because of a house fly's behavior and survival needs, it frequently comes in contact with pathogenic organisms. House flies are extremely capable of transmitting pathogens mechanically (West 1951). West (1951), and most recently Greenberg (1973), have compiled extensive lists of the pathogens (bacteria, viruses, fungi, protozoa, and nematodes) the house fly is capable of transmitting. The transmission of *Campylobacter spp.*, E. *coli* O157:H7, *H. pylori, C. parvum*, and *G. lamblia* are probably the most significant pathogens capable of being transmitted by the house fly recently reported (Shane et al. 1985, Grubel et al. 1997, Kobayashi et al. 1999, Graczyk et al. 2003).

Of particular importance is the exponential proliferation of house flies following situations arising from natural disasters or conflict. Following the tsunami that devastated parts of Indonesia in 2004, sewage and drainage systems were destroyed leaving sewage pools that bred numerous species of filth flies (Burrus 2005). This is often what occurs following any of these chaotic events; communities are left in shambles and multiple oviposition sites develop due infrastructural collapse or basic municipal sanitation being out prioritized. Large fly populations

then develop and epidemic levels of diarrheal cases normally follow (Thornton et al. 2005, Watson et al. 2007). This reduces military readiness and stresses health care systems (Putnam et al. 2006). The direct impact of house flies on disease transmission in these situations is either not measured or often not measurable because the same pathogens transferred by house flies can be just as easily transferred by man or other organisms.

Control

The house fly is best controlled through a fly management program based on the sound principles of Integrated Pest Management (IPM), including a combination of monitoring, cultural, biological, and chemical control measures (West 1951, Keiding 1976). Monitoring is any technique employed to determine presence/absence and peak/trough flows of house fly populations. Cultural controls are any measures that deliberately alter the life cycle of the house fly without the use of chemical or biological agents. Biological controls are agents or organisms that alter the life cycle of the house fly and chemical controls are naturally- or synthetically-derived chemicals that can alter the life cycle of the house fly.

Every aspect of the fly management program should have some form of fly monitoring to determine when and what type of approach should be employed (pre-treatment survey) and to see the effectiveness of the treatment (post-treatment survey) (Keiding 1976). In the pre-treatment survey, house fly density, distribution, and behavior should be noted to help determine which treatment option to use. Post-treatment surveys normally only need to monitor fly densities – unless failure occurred. In this case, a complete reassessment should be done to include some form of monitoring for insecticide resistance.

There are four basic methods to obtain a fly population index: counting flies, counting fly specks, netting flies, or trapping flies (Keiding 1976). If counting adult flies, several methods can be used. The classic technique is the use of a Scudder grill – a simple grid constructed of

wood that can be placed over an infestation source and then all flies landing on the grid are counted (1947). A similar technique that can be used in practically any situation is to simply mark an area (preferably near infestation or resting areas) and count flies landing on it. Counting fly specks left on index cards may be the method of choice today for indoor sampling because of its simplicity. Spot cards can be positioned in standard locations throughout the infested area and will give a good representation of the fly populations over time. Simply hang them and check back on them after a designated time period. Over time it will show peak and troughs of fly populations; in addition, the spot cards can be archived and marked with any insecticide treatment used to provide additional documentation for resistance monitoring. Some users, however, prefer to use destructive sampling. In these cases, baited traps, sticky ribbons, or even netting flies can work well.

All of the above methods work and can give consistent numbers indoors as long as the same sampling method is used for all counts. However, infestations that occur outdoors are not as easily monitored. With outdoor sampling, spot cards are an unrealistic method because placement would be difficult and precipitation would destroy them. Scudder grills and other fly count techniques are subject to wide variation depending on positional effects, time of day, and weather conditions (Geden 2005). Baited traps and net sweeping may be the best techniques to use outdoors for surveillance work, but these methods are destructive and not suitable in every situation. Beck and Turner (1985) found that using a simple visual index correlated better with absolute fly densities than spot cards, sticky ribbons, scudder grill and fly counts, but these indexes are very subjective and will vary between persons making the counts. Perhaps, the best way to monitor house flies outdoors is to use a combination of the methods described.

By far the most important aspect of a fly management program is the use of cultural controls. Cultural controls target the breeding and feeding sites of adult and larval flies. Additionally, they are used to prevent adult flies from contacting food, pathogens, and man (Keiding 1976). Garbage is the main source of infestation in an urban environment and manure is the main source in an agriculture environment, however, both sources can be found in any environment. In developed urban communities, these breeding sites are normally controlled by very established municipal sanitation measures (e.g., closed sewers, garbage removal, etc.) (Hogsette 1995). Agriculture facilities have to physically remove manure or bake it by covering dung heaps with plastic sheeting. Garbage should be removed from the area at least twice weekly or burned (Keiding 1976). Windows, screens, and doors should all be in good repair and kept closed to prevent flies from entering establishments. The installation of air curtains on doors and windows that are frequently opened and closed is an energy efficient option that can reduce the number of flies that enter.

Traditionally baited traps, light traps, electrocuting light traps, and sticky ribbons have been used as cultural control measures, but their effectiveness at reducing house fly populations are limited and their use should primarily be considered as a monitoring technique. However, these methods do trap and kill flies so using them should never be automatically ruled out. In fact, if the likelihood of large infestations does not exist, then traps are a good method for killing flies (such as in grocery stores and restaurants) but some considerations should be made prior to their use. Baited traps generally can not be used indoors or near residences because the odor associated with this method is repulsive to humans (Pickens et al. 1973). Electrocuting light traps release fly parts, bacteria, and viruses and may be just as unhygienic as the fly itself and should not be used in areas were conditions need to remain relatively aseptic (Urban and Broce

2000). Lighted traps need bulb replacement approximately every six months, and sticky ribbons need replaced frequently due to dust and fly cadaver build-up. Flies that do make their way into an establishment can be physically removed by numerous devices, but the most common physical cultural control method is the good 'ol fashioned flyswatter. A novelty gadget used for killing insects, including flies, has recently sparked numerous videos on the World Wide Web. The device is a combination electrocuting trap and fly swatter. This device may be interesting and fun, but the same risk is associated with it as the regular electrocuting light traps.

The second most important principle of a good fly management program is the use of biological controls. Every stage of a fly's life cycle is vulnerable to attack by some form of biological control (West 1951). The eggs are often predated upon by mites, earwigs, ants, and some beetles. Larvae are also attacked by mites, earwigs, and beetles, as well as some birds, wasps, and other Dipteran larvae. Pupae are often parasitized by small wasps and some beetles, others can be eaten by birds and large beetles. Many adult house flies meet their demise thanks to predatory insects (mantids, flies, dragonflies, wasps, ants) and arachnids. Many other adults are eaten by reptiles, amphibians, small mammals, and birds. House flies are also prone to infections by bacteria and fungi. Fortunately, all of these biological controls are already abundant in a fly's natural environment and the goal of a fly management program should be to maintain or supplement these existing populations (Geden 1995). Parasitic wasps can now be purchased commercially and their successful use is variable (Axtel 1999).

The use of chemical insecticides is the third component of a good fly management program. Chemical insecticides provide quick results (i.e. dead flies) and satisfactory control in one or two days, but their use should be limited to reduce the likelihood of resistance evolution and maintenance of non-target organisms. Chemical insecticides can be applied several different

ways: residual surface treatments, larviciding, space sprays (including aerial spraying), and baits. The most common method for fly control is the use of space sprays and dry insecticidal baits.

Residual surface treatments can be applied to any surface in any location the label allows but they are most effective when applied directly to fly resting areas (Keiding 1976). Neglecting to monitor and treat areas where flies are primarily resting will result in excess insecticide usage and population reductions of non-target organisms. Several insecticides are available for residual treatments; most are organophosphate based. All are generally good for long term control, but there excessive use may increase selection for insecticide resistance. One technique for residual insecticide application is the use of insecticide-impregnated cords which target the distinctive behavior of flies to rest on objects with edges (Scudder 1949, Keiding 1965). This method is thought to be less likely to select for resistance because the treated area is small and treatments can be readily removed or replaced with additional cords treated with insecticides from different chemical classes (Appendix A).

Larviciding with insecticides sounds great in theory because larvae are relatively non-mobile compared to the adult flies, which have the ability to fly to different areas if one is found unsuitable; however, larviciding is really not a practical method for extended control. Larvicides have to be applied frequently because they are applied to areas such as garbage and manure - both of which constantly accumulate. Larvicides kill non-target organisms coming in to feed on the house fly immature stages and would reduce those populations over time. In addition, if the same class of insecticide is used for larviciding that is used for adult control resistance selection would be rapid. Larvicides are effective if sites to be treated are expected to exist for a short period of time; in these instances several organophosphate insecticides and insect growth regulators can be used.

Space sprays are primarily pyrethrin- or pyrethroid-based insecticides, but some may also be organophosphate-based, that are sprayed into the air in a fly infested space or over a fly infested area. These types of insecticides target the fly nervous system and cause rapid knockdown of contacted flies (Yu 2007). Space sprays are more effective when an abundance of flies are concentrated in one area; this occurs mainly in the evening indoors and in the morning outdoors (Keiding 1976). They have little to no residual and have to be reapplied frequently (daily) in areas of large infestations. Another type of space treatment is made through the use of insecticide vaporizers. The only vaporizer currently available is formulated with the organophosphate dichlorvos, but its use is becoming more restricted and its future longevity may be short lived.

Insecticide baits are easy-to-use insecticides that have added attractants into the formulation matrix to draw flies into the treated area to contact the insecticide either by ingestion or contact. A basic bait matrix is a simple solution of sugar, water, and an insecticide. Complex bait matrices contain multiple sugars, pheromones (Z-9-tricosene), and other substances found attractive to house flies. The most widely used fly baits available are formulated in dry granules as scatter baits containing carbamates and neonicotinoids (Appendix B), however, other bait products are available and frequently used. Like space sprays and larvicides, baits have to be frequently applied because environmental conditions degrade them or they become covered by manure or garbage. Also, when baits are used in areas with large fly populations, the flies will consume the bait rapidly leaving little bait behind for the immature stages that will eventually emerge.

CHAPTER 3
INSECTICIDE-IMPREGNATED CORDS FOR HOUSE FLY CONTROL

Introduction

The house fly, *Musca domestica* L., is widely considered the most common nuisance pest. Their nuisance pest status can quickly change to a public health risk if fly populations occur near inhabited areas where pathogen-rich oviposition sites are found. Areas stressed due to natural disasters, humanitarian crises, or combat are often plagued by large fly populations (Rosales and Prendergast 2000, Burrus 2005, Thornton et al. 2005). The most effective way to control house flies and reduce the risk of disease transmission is by eliminating their pathogen-rich oviposition sites. Oviposition site removal may be impractical, especially in areas affected by natural disasters and combat, where the oviposition sites are too numerous or difficult to reach.

The best control method to use when sanitation fails or when fly populations need to be rapidly controlled are chemical insecticides. Chemical insecticides provide rapid kill of house flies and markedly reduced fly densities can be achieved in as little as 1-2 days. Baits and space sprays are the primary chemical insecticide methods used for house fly control today, but both methods provide little or no residual control, and resistance to their active ingredients is well documented in house flies (Georghiou and Lagunes-Tejeda 1991, Liu and Yue 2000, Scott et al. 2000). In addition, the number of registered insecticides available for house fly control in the United States continues to decrease (Kaufman et al. 2001). New insecticides and application methods are clearly needed to avoid future insecticide resistance problems.

Insecticide-impregnated cords have been used with great success to control flies and are considered less likely to select for resistance than traditional residual sprays (Keiding 1976). Their first use was in 1947 (Baker et al.) and by the mid-1950's, insecticide-impregnated cords were commercially available and widely used (Fehn 1958, Smith 1958). The commercially

available cords contained 13.79% parathion and 3.54% diazinon (Smith 1958). Cords impregnated with high concentrations (up to 25% active ingredient) of other organophosphate and organochlorine insecticides were also widely used with great success (Kilpatrick and Schoof 1959, Keiding 1976, Rabari and Patel 1976). These products are no longer used today due to the popularity of insecticidal baits and space sprays and because the Environmental Protection Agency, acting under federal legislation, eliminated the use of their active ingredients.

The objective of this study was to investigate if cords impregnated with newer insecticides would be an effective tool for house fly control. Specifically, the objectives were to: 1) determine the attractiveness of various natural and synthetic cords to house flies, 2) determine the effectiveness of fipronil and indoxacarb on the most attractive cord materials, and 3) evaluate the effectiveness of the best cord/treatment combination in a simulated field environment.

Materials and Methods

Insects. The Horse Teaching Unit (HTU) strain of house flies, *M. domestica* L., reared at the University of Florida in Gainesville was used for all experiments. Larvae were reared on a diet medium, modified from Hogsette (1992), containing 3 liters wheat bran, 15 ml methyl paraben, 1.5 liters water, and approximately 200 g (250 ml) dairy calf feed (Calf Manna® pellets, Manna Pro Corp., St. Louis, MO). All developmental stages were held at 26 ± 1°C and 55% RH with a 12:12 (L:D) photoperiod. Adult flies emerged within screened rearing cages and were provided granulated sugar, powdered milk, and water *ad libitum*.

For all assays, adult house flies (3-5 d old) were aspirated from the screened rearing cages using a handheld vacuum with a modified crevice tool attachment. Flies used for the laboratory assays were placed into a 5°C environment for 5 min to subdue activity. Flies were then placed on a chilled aluminum tray, sexed, and counted. House flies used for field cage assays were not

anesthetized, but were aspirated from the screened rearing cages and released directly into field cages.

Laboratory Arenas. Arenas (31 x 25 x 21 cm) were constructed using PVC pipe (1.27 cm [0.5 in]) (Figure 3-1A). Rubber bands were used to establish individual treatment positions; four treatment positions were used in the cord attractiveness bioassay and five positions were used in the impregnated cord bioassay. All cords were attached to the treatment positions vertically using paper clips and were uniformly distributed along the length of the arena. The cord attractiveness bioassay held two randomly assigned cords at each treatment position and the impregnated cord bioassay held only one cord at each treatment position according to a 5 x 5 Latin square configuration. Arenas were enclosed with a transparent plastic bag (3716 cm2 [24 x 24 in], 1 mil poly, Uline, Waukegan, IL).

Cord Attractiveness Bioassay. Eight cords were evaluated: nylon (Braided, Multi-Purpose Braid 75 lb. load limit, Wellington Cordage LLC, Madison, GA), polypropylene (Braided, Multi-Purpose Rope – 56 lb. load limit, Wellington Cordage LLC, Madison, GA), cotton (Braided, Multi-Purpose Sash Cord – 28 lb. load limit, Wellington Cordage LLC, Madison, GA), cotton wick (Sterilized roll, #200209, Richmond Dental Company, Charlotte, NC), manila (Twisted, Natural Rope – 108 lb. load limit, Wellington Cordage LLC, Madison, GA), wool (Twisted, Natural Cord, Wooded Hamlet Designs, Greencastle, PA), leather (Tan laces, #6192, Rothco, Ronkonkoma, NY), and parachute cord (550 test, white, purchased locally from M & C Army Surplus Store, Gainesville, FL).

Fifty female flies were released into each arenas and 10% sugar water was provided *ad libitum*. Number of flies resting on cords was counted every 10 min for 2 hr. Arenas were lightly shaken between each count to displace flies from their resting positions. Four replications

were performed in the laboratory (28 ± 1ºC) under continuous light on separate days using different flies.

Impregnated-Cord Laboratory Bioassays. Cotton, manila, wool, polypropylene, and nylon cords were selected from the cord attractiveness experiments to be evaluated in the impregnated-cord experiments. Each impregnated-cord experiment consisted of 6 arenas. Five arenas were organized into a 5 x 5 Latin square design, blocking for treatment position, and a sixth arena was used as a control. The control arena had no treated cords and all cords within it maintained the same cord positions throughout all experiments (left to right: position 1 = cotton; 2 = wool; 3 = manila; 4 = polypropylene; 5 = nylon).

Separate experiments were done to evaluate cords (15.24 cm length [6 in], 0.6 cm [0.25] diam) impregnated with a 0.1% fipronil or a 0.6% indoxacarb solution. The 0.1% fipronil solution was prepared by combining 2.7 ml of the formulated insecticide (Termidor SC, 9.1% a.i., BASF, Research Triangle Park, NC) with 250 ml of tap water. The 0.6% indoxacarb solution was prepared by combining 5 g of formulated insecticide (DPX MP062, 30WG, DuPont, Wilmington, DE) with 250 ml of tap water. Cords were impregnated by dipping for ~2 sec in the insecticide solution and were then allowed to dry in a fume hood.

Groups of 50 female flies were placed within each arena and provided a 10% sugar water solution *ad libitum*. Mortality counts were recorded until at least 80% mortality was observed. Due to the differences in the mode of action of the insecticides, mortality for flies exposed to fipronil-impregnated cords was defined as the inability to remain standing; flies exposed to indoxacarb-impregnated cords were considered dead if they were unresponsive to touch. Each experiment was run in the laboratory (28 ± 1ºC) under continuous light and replicated twice.

Impregnated-Cord Field Cage Bioassay. Cages (1.8 x 3.7 x 1.8 m) were constructed from PVC pipe (2.54 cm [1 in] diam) and enclosed with mesh screening (Outdoor Cage, #1412A, 18 x 14 mesh, Bioquip, Rancho Dominguez, CA). Black plastic sheeting (6 mil) was used to line the floor. A sampling stage, constructed of two vertical cinder blocks and an inverted storage bin (Palletote #1721, 37 liter, Rubbermaid, Winchester, VA), was placed in the center of the cage (Figure 3-1B). On top of the sampling stage there were two 994-ml (1 qt) chick waterers, one filled with 10% sugar water and the other with tap water, and a 60-ml plastic cup filled with 8 g of previously used larval house fly medium. The chick waterers provided enough sustenance for the duration of the test and the plastic cup was used as an attractant. The plastic cup was covered with a paper towel and sealed with a rubber band to prevent flies from ovipositing on the medium.

Treatments consisted of two long (0.9 m) and eight short (0.6 m) lengths of 0.1% fipronil- and 1.2% indoxacarb-impregnated wool cords. The 0.1% fipronil solution was prepared by combining 7.7 ml of the formulated insecticide (Termidor SC, 9.1% a.i., BASF, Research Triangle Park, NC) with 700 ml of tap water. The 1.2% indoxacarb solution was prepared by combining 28 g of formulated insecticide (DPX MP062, 30WG, DuPont, Wilmington, DE) with 700 ml of tap water. Each cord was treated in the same manner as the laboratory experiments, except the cords were dipped and soaked for 1 min prior to drying.

Depending on fly availability, 27.5 – 35 ml (9.8 ± 1.8 flies/ml) of flies was released into each cage. After a 1-h acclimation period, pre-treatment fly counts were taken. Before fly counts were taken, the operator walked three laps around the interior of the cage to disturb flies from their resting positions and to recover any dead flies from the cage floor. Four consecutive fly counts were then taken from the outside of the cage 1 min after exiting. All flies that landed

on the sampling stage, chick waterers, and plastic cup attractant were counted. Treatments were then hung vertically from the mesh ceiling using paper clips in specific locations (Figure 3-1C) and post-treatment fly counts were taken at 24 and 48 h using the same method described above. After the initial 48 h evaluation, treatments were aged in the elements for four weeks, at which point residual effectiveness was re-evaluated as described above. Three replicates were performed at each treatment age (0 and 4 wk).

Statistical Analysis.

All statistical analyses were performed using JMP IN (SAS Institute 2005), except probit analysis estimates were performed using SAS (SAS Institute 2001). For the cord attractiveness experiments, the mean number of flies/cord was analyzed using a one-way analysis of variance and contrasts were performed between natural and synthetic cords and the animal- and plant-based cords. For the laboratory insecticide-impregnated cord laboratory experiments, mortality data were corrected using Abbott's formula (1925) and arcsine square root-transformed. A two-way analysis of variance was performed on the 24-h fipronil data and the 48-h indoxacarb data to determine if treatment position had an effect on mortality. LT_{50} values were estimated by probit-analysis regression (Finney 1971). Potency ratios, using the cotton cord as the standard, were performed using the method described in Robertson and Preisler (1991). Slopes, LT_{50} values, and potency ratios were considered significantly different if the 95% confidence intervals did not overlap. For the field cage experiments, percent fly count reductions were calculated from the control fly counts. Fly count reductions and mortality data (number of dead flies recovered from cage floor) were then analyzed for each treatment age (0 and 4 wk). All means were separated using the Student's T or Student-Newman-Keuls test ($\alpha = 0.05$).

Results

In the laboratory studies, all flies fully recovered from chilling after approximately 45 min at which point the flies were dispersed throughout the entire arena. Flies were more attracted to the manila cord, which had significantly more flies resting on it than any other cord (Figure 3-2). No significant differences were seen between the other natural cords or between the synthetic cords; however, all synthetic cords had significantly less flies resting on them than the natural cords (F: 112.69, df = 368, P = <0.001) and the plant-based cords were more attractive than the animal-based cords (F: 11.64, df = 368, P = <0.001). The least attractive cord was the nylon parachute cord.

The laboratory design had no position or interaction effects for either fipronil (F: 1.05; df = 4; P = 0.3982, F: 0.8347; df = 20; P = 0.6583) or indoxacarb (F: 0.71; df = 4; P = 0.5906, F: 0.32; df = 20; P = 0.9955) in the insecticide-impregnated cord experiments. At the 24 h (fipronil) and 48 h (indoxacarb) recordings, all impregnated-cords had significantly higher mortality than the controls (Figure 3-3).

House flies suffered significantly higher mortality when exposed to the fipronil-impregnated wool cord than any other fipronil-impregnated cord at 24 h (93%). The other fipronil-impregnated natural cords had percent mortalities below 15%, with manila causing only 5% mortality at 24 h. No significant differences in mortality were seen between the fipronil-impregnated nylon and polypropylene cords or the fipronil-impregnated cotton and manila cords at 24 h.

The indoxacarb-impregnated wool cord caused significantly higher mortality (85%) than any of the other cords except for the cotton cord at the 48 h recording. No significant differences in mortality were seen between the synthetic indoxacarb-impregnated cords or between the cotton and manila indoxacarb-impregnated cords. Significant differences in mortality were seen

between the wool and manila indoxacarb-impregnated cords. The indoxacarb-impregnated nylon cord caused the lowest mortality at 48 h (47%).

Fipronil- and indoxacarb-impregnated cords efficacy results can be viewed in Table 3-1. In general, the fipronil impregnated cords had lower LT_{50} and LT_{90} values than the indoxacarb-impregnated cords. Among the fipronil-impregnated cords, the wool cord had the lowest LT_{50} and LT_{90} values and the impregnated cotton cord had the highest LT_{50} and LT_{90} values. The LT_{50} values for the synthetic cords were relatively low compared to the other cords, but the LT_{90} values were no different from the cotton cord. The manila cord LT_{50} value was the second highest, but had the second lowest LT_{90} value behind the wool cord; it is important to note that it also had the highest slope compared to the other cords. All cords were more effective than the cotton cord except for the nylon cord's LT_{90} value.

All indoxacarb-impregnated cords had LT_{50} values >32 h and LT_{90} values >51 h. The indoxacarb-impregnated wool cord had lower LT_{50} and LT_{90} values than all other indoxacarb-impregnated cords except for the manila cord, which showed no significant differences in LT_{50} values. The indoxacarb-impregnated polypropylene and cotton cords each had LT_{50} values of 52 h and LT_{90} values >100 h, which were the highest values for the experiments. No differences in LT values were observed between the manila and nylon cords. All cords were more effective than the cotton cord except for the polypropylene cord's LT_{50} and LT_{90} values.

In the field cage experiments, no significant differences in fly count reductions occurred between the treatments (Table 3-2). Both treatments had >57% fly count reductions by 24 h and >87% by 48 h, independent of the treatment age. Dead flies were collected from all cages at every recording; significantly more were collected from the treatment cages than the controls. Fipronil treatments had significantly more dead flies than the indoxacarb treatments with fresh

cords at 24 and 48 h and with aged cords at 24 h. No significant differences were seen between the number of dead flies collected from the fipronil treatments and indoxacarb treatments at the 4 wk, 48-h recording.

Discussion

Insecticide-impregnated fly cords are based on a fundamental component in a fly's behavior – flies prefer to rest on objects with distinct edges, such as twigs, wires, cord, and line (Scudder 1949). Since insecticide-impregnated cords only represent a small proportion of available resting surfaces available to flies, it is assumed that factors which enhance a fly's attraction to the cords would be beneficial to the effectiveness of the treatment. Surfaces which are more attractive to flies would be expected to cause quicker mortality because of increased exposure to the insecticide. Arevad (1965) found flies to favor dark, rough surfaces over light, smooth surfaces. Specific factors influencing a fly's attraction to natural fiber cords were evaluated by Fay and Lindquist (1954). They found sisal cord to be more attractive than jute or wool cords of the same size, but less attractive than a similar sized cotton cord. When given a choice between only cotton and sisal cords, flies preferred the sisal cord. They also found that the same type of cord was more attractive to flies as the cord diameter increased between 0.13-1.1 cm.

In our attractiveness experiment, the cords we evaluated varied by fiber type (animal or plant), color, texture, and, in some cases, even diameter. All of the natural cords we evaluated were more attractive than the synthetic cords. The natural cords were "rougher" than the relatively smooth synthetic cords; in addition, the plant-fibered manila cord and the animal-fibered leather cord were darker than the other cords. These factors may have increased their overall attractiveness to the flies. If comparing the most attractive cord (manila) to the least attractive (parachute cord) the differences in texture and color are substantial (Figure 3-4).

Manila is a very rough, coarsely textured brown thatch cord made from the leaf fibers of the abaca tree, *Musa textiles,* while the parachute cord is a relatively smooth kernmantle cord made of white nylon. The parachute cord was one of two cords less than 0.64 cm, which may have decreased its attractiveness. The other cord less than 0.64 was the leather cord, but it was as attractive as the other natural cords (except manila) despite its diameter being half the size. The leather cord's dark color may have increased its attractiveness or it may have been more attractive due to animal odors that were still associated with the material.

The previously available commercial fly cords were exclusively made of cotton. Cotton was cheap, durable, absorbent and widely available. Although cotton was relatively attractive in our experiments, it had very poor efficacy for both fipronil and indoxacarb when compared to the other natural and synthetic cords tested indicating that it may not be the best type of cord to use for insecticide treatment. Fipronil- and indoxacarb-impregnated wool cords had the greatest efficacy in our experiments despite flies resting on it 50% less than the manila cord in the cord attractiveness experiments. This is contrary to the previous assumption that quicker mortality would result from increased exposure to a more attractive insecticide-impregnated cord and neglects to account for the insecticide-substrate interaction. Highly organic materials readily bind to pesticides and make them less effective (Dell et al. 1994, Gardner et al. 2000) and may have accounted for the low LT_{50} and LT_{90} values seen in the cotton cords and in the LT_{50} value of the fipronil-impregnated manila cord. The exact reason wool outperformed the other cords in our experiments was not fully investigated, but it is likely due to the insecticide-substrate interaction. The wool cord was the only animal-fibered cord evaluated and is naturally impregnated with several oils. Both fipronil and indoxacarb are very lipophilic insecticides and

probably dissolved readily within these oils which likely increased the rate of insecticide transfer from the cords through the waxy layers of the fly's cuticle.

In the laboratory, the indoxacarb cords generally provided a much slower kill than the fipronil cords, however, in the field cages differences were not as apparent. Indoxacarb is a pro-insecticide that needs to be bioactivated within the insect before it is toxic and will always cause mortality slower than an insecticide, such as fipronil, that is toxic upon contact once a lethal dose is obtained. Flies poisoned by indoxacarb in the laboratory are shielded from desiccation and predation, which may have proved to be vital to their prolonged survival in the laboratory experiments. Furthermore, the indoxacarb dose was increased in the field cage experiments and may have affected the faster results seen in the field cage experiments. Both treatments showed a decrease in efficacy in the field experiments after being aged 4 weeks, but still had adequate fly count reductions and causing significantly more flies to die than the control.

In conclusion, the use of insecticide-impregnated cords is very practical to supplement a house fly management program. Insecticide-impregnated cords ensure adequate residual coverage in areas difficult to treat with traditional residual insecticides and they can easily be removed and relocated to other fly resting areas if needed or alternated with cords impregnated with other active ingredients to reduce the possibility of resistance development. More research still needs to be done to determine adequate doses and rates of treatment, keeping in mind that these may vary depending on cord type and insecticide used. Wool cord outperformed all other cords evaluated in this study and fipronil and indoxacarb both appear to be effective insecticides for house fly control.

Table 3-1. Efficacy of various cords impregnated with 0.1% fipronil or 0.6% indoxacarb on female house flies.

				Lethal Times (h) (95% CL)[§]				Potency Ratio (95% CL)[§]	
Treatment	Cord	*n*[†]	Slope ± SE[§]	50	90	χ^2	P	50	90
Fipronil	Cotton	2750	9.52 ± 0.36b	39.7 (39.2-40.2)e	54.1 (52.9 - 55.5)c	9.060	0.1067	1.00 e	1.00 d
	Manila	1000	12.98 ± 0.83a	35.0 (34.5-35.6)d	44.0 (42.6 - 45.7)b	1.213	0.5445	1.13 (1.12-1.15)d	1.23 (1.21-1.25)b
	Wool	1250	5.32 ± 0.29c	12.9 (12.3-13.4)a	22.4 (21.3 - 23.8)a	0.650	0.4200	3.09 (3.01-3.16)a	2.42 (2.35-2.48)a
	Polypro	2500	4.65 ± 0.29d	26.2 (25.6-27.0)c	49.6 (46.4 - 53.8)c	3.643	0.7249	1.51 (1.45-1.57)c	1.09 (1.04-1.14)c
	Nylon	1500	3.68 ± 0.25e	23.0 (21.2-24.6)b	51.3 (48.2 - 55.4)c	1.455	0.6927	1.72 (1.66-1.79)b	1.05 (1.00-1.11)cd
Indoxacarb	Cotton	2248	4.04 ± 0.13c	52.2 (50.3-54.3)c	108.5 (102.1-115.9)c	1.3494	0.5093	1.00 c	1.00 c
	Manila	1659	5.10 ± 0.74b	36.2 (32.3-38.7)ab	64.7 (60.2 - 73.4)b	3.4030	0.3336	1.44 (1.35-1.54)ab	1.68 (1.54-1.82)b
	Wool	4250	6.44 ± 0.16a	32.6 (32.0-33.1)a	51.5 (50.1 - 53.1)a	8.6295	0.2804	1.60 (1.54-1.67)a	2.11 (2.01-2.20)a
	Polypro	2250	3.11 ± 0.20d	52.2 (49.7-54.5)c	134.8 (122.7-151.7)d	0.6717	0.7147	1.00 (0.91-1.10)c	0.80 (0.72-0.90)c
	Nylon	2000	6.57 ± 0.54a	39.2 (37.2-40.8)b	61.5 (59.4 - 64.4)b	2.9321	0.2308	1.33 (1.27-1.39)b	1.76 (1.68-1.85)b

[†] Total number of trials; 500 flies/trial except for the cotton (498) and manila (487) indoxacarb-impregnated cords (Probit [SAS Institute 2002]).

[§] Mortality was corrected using Abbott's Formula. Means within a column, in the same treatment group, followed by the same letter are not significantly different based on non-overlap of 95% confidence intervals.

Table 3-2. Cumulative number of dead flies and percent fly count reduction in relation to control fly counts of house flies exposed to 0.1% fipronil- and 1.2% indoxacarb-impregnated cords in field cages.

		% Fly Count Reduction ± SEM‡		# of Dead Flies†‡	
Treatment Age	Treatment	24 h	48 h	24 h	48 h
0 Weeks	Fipronil	80.22 ± 13.10a	98.66 ± 1.34a	83.0 ± 1.0a	95.3 ± 3.4a
	Indoxacarb	57.39 ± 6.92a	97.21 ± 1.43a	30.3 ± 4.4b	59.7 ± 2.0b
	Control			4.7 ± 4.2c	11.3 ± 8.5c
4 Weeks	Fipronil	59.25 ± 22.10a	87.43 ± 12.57a	53.0 ± 7.8a	79.3 ± 7.2a
	Indoxacarb	64.39 ± 5.89a	87.72 ± 7.34a	30.3 ± 4.7b	62.0 ± 8.1a
	Control			3.3 ± 1.7c	10.3 ± 2.0b

† Cumulative mean number of flies recovered from cage floor.

‡ Means in a column, within the same treatment age, followed by the same letter are not significantly different ($P > 0.05$; Student's T or Student-Newman-Keuls test)

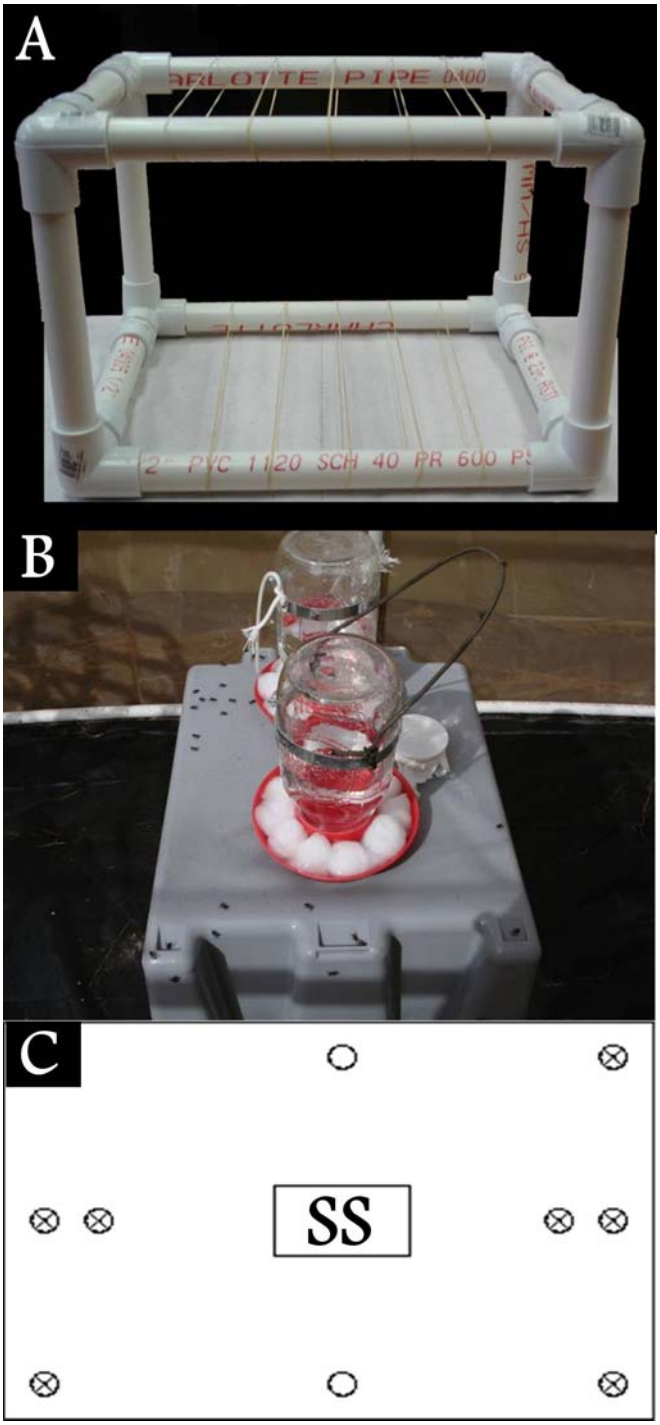

Figure 3-1. Laboratory and field experimental design elements. A). Laboratory arena constructed of PVC pipe. Cords were suspended between the rubber bands using paper clips. B) Sampling stage used in field cage experiments. Chick feeders with either 10% sugar water or tap water and a plastic cup containing previously used larval medium was used as sustenance and attraction. C). Cord placement in relation to sampling stage (SS) in the field cage bioassay. Crossed circles were short cords (0.6 m) and empty circles were long cords (0.9 m).

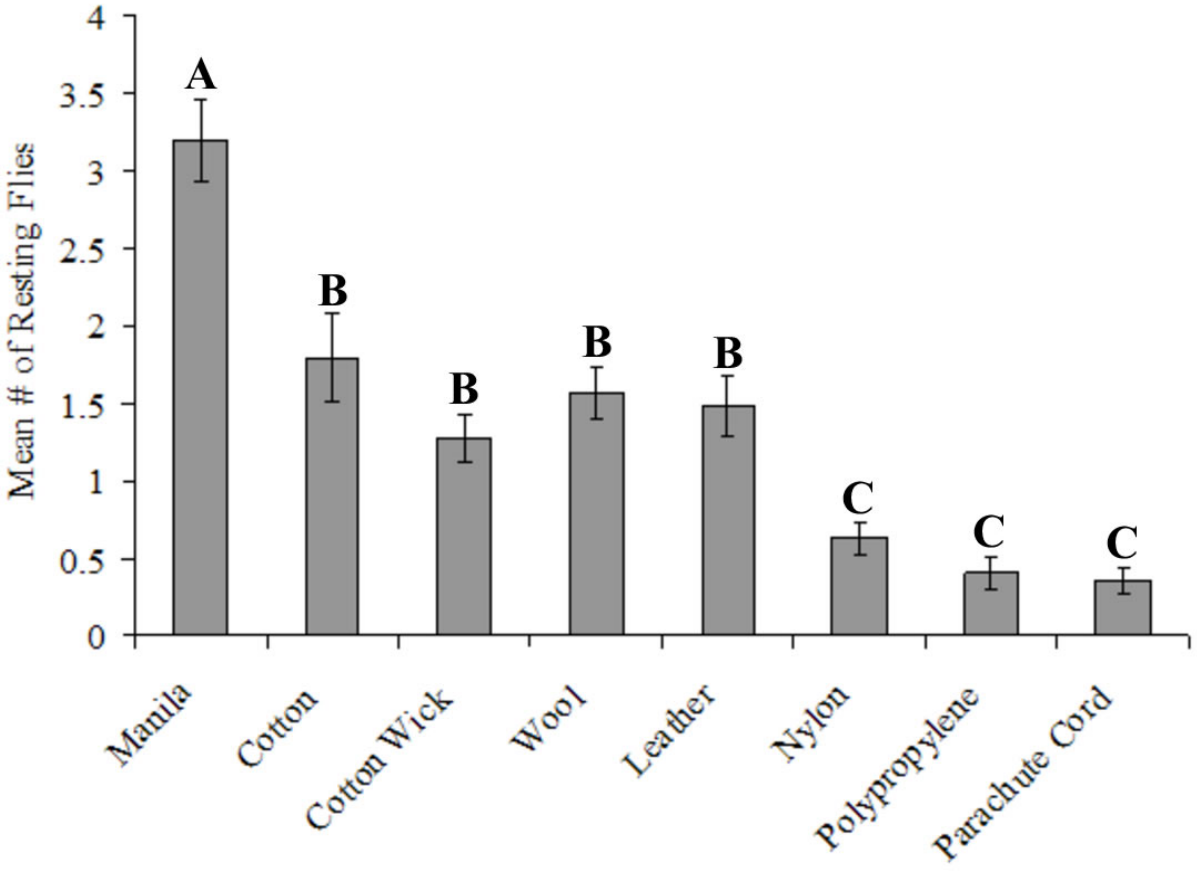

Figure 3-2. Attraction of female house flies to various natural and synthetic cords.

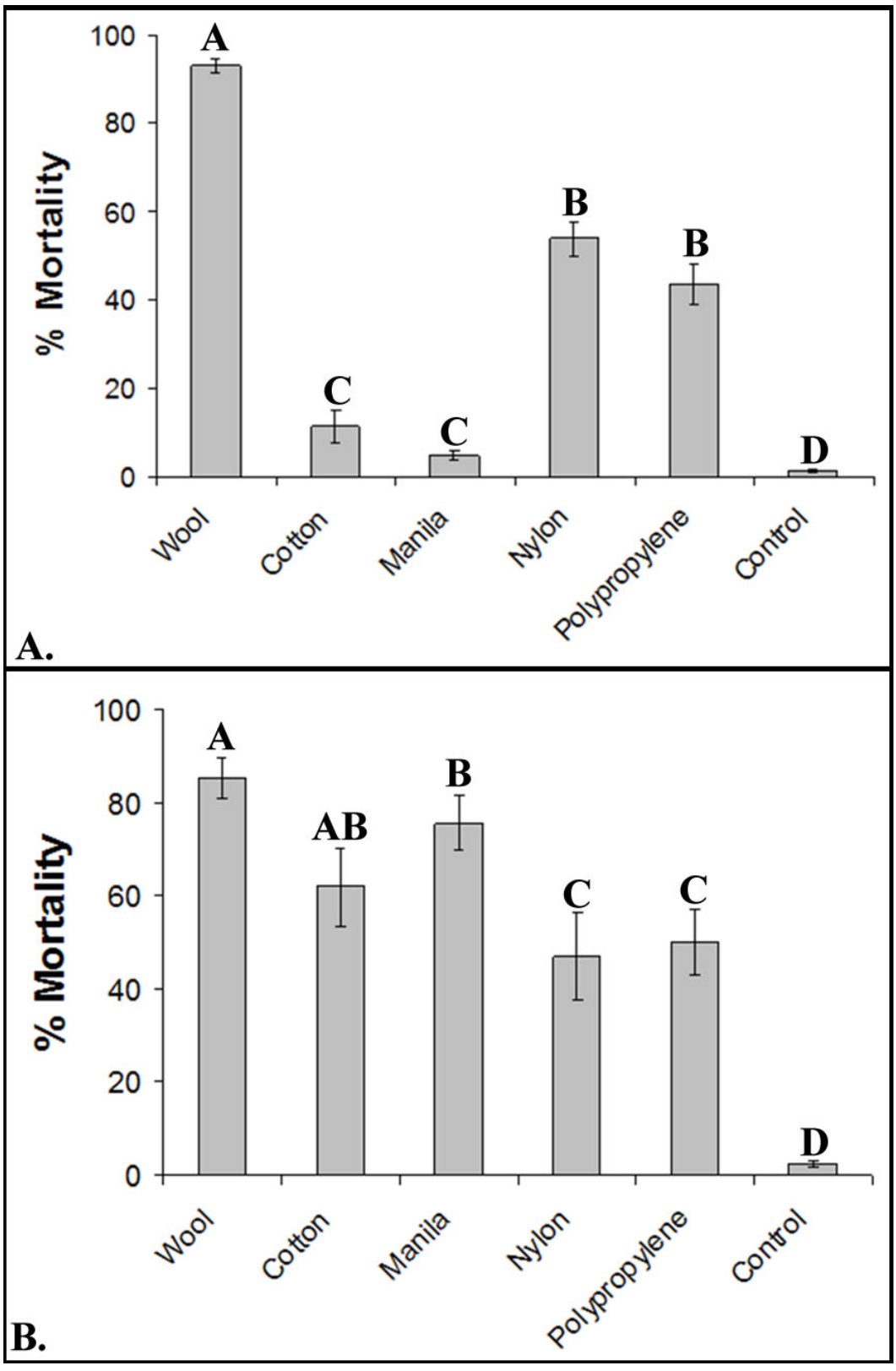

Figure 3-3. Female house fly mortality exposed to various natural and synthetic cords treated with 0.1% fipronil for 24 h (A) and 0.6% indoxacarb for 48 h (B).

Figure 3-4. Comparison of the most attractive cord (manila) and the least attractive cord (nylon parachute) in the cord attractiveness experiments.

CHAPTER 4
EVALUATION OF A NEW IMIDACLOPRID BAIT FOR HOUSE FLY CONTROL

Introduction

The house fly, *Musca domestica* L., is the most commonly encountered pest of the generalized group of Diptera called filth flies. Large numbers of house flies are frequently found in areas where manure, garbage, and other decaying organic matter are abundant. Although primarily a nuisance to people and animals, house flies can pose a health risk by mechanically transferring pathogens picked up from their breeding sites, particularly when they enter homes or eating establishments. When the source of infestation is inaccessible or sanitation measures are not effective, house fly control is often achieved using dry insecticidal scatter baits.

Two widely used scatter baits are Maxforce® Granular fly bait and Golden Malrin® fly bait. Maxforce® Granular is an imidacloprid-based bait containing the fly attractant (Z)-9-tricozene, the bittering agent Bitrex®, and other attractants and inert ingredients. It is currently the only imidacloprid-based scatter bait available. Golden Malrin® contains 1.1 % methomyl, 0.049 % (Z)-9-tricosene, as well as other attractants and inert ingredients, and is one of several methomyl-based scatter bait formulations available.

In general, dry scatter baits have many advantages over other types of insecticidal fly control products: they are easier to work with in field environments, they can be more attractive to flies than liquid baits, and they usually have a longer storage shelf life (Gahan et al. 1954, Darbro and Mullens 2004). However, dry scatter baits need to be replaced frequently in some areas when granules become covered by manure or other debris (Barson 1987). The U.S. Environmental Protection Agency (EPA), acting under legislative mandates, also requires the scatter bait granules be dyed to distinguish them from other non-toxic materials. For example, the Maxforce® Granular fly bait is formulated as red granules and the Golden Malrin® is

formulated as blue granules. When these granules become wet, the dye often bleeds onto the surrounding surface and may be unsightly for the user.

Label restrictions are also very different and can limit the uses of certain active ingredients or insecticide products. Golden Malrin® can only be applied as scatter bait or within bait stations, whereas, the Maxforce® Granular can be applied as scatter bait, within bait stations, or it can be mixed with water and painted onto surfaces allowing it to be applied directly to distinct fly resting areas, such as on ceilings or rafters. However the use of Maxforce® Granular is more restricted than that of Golden Malrin® because its label restricts its use in food establishments. Golden Malrin®, despite being the only carbamate-based insecticide not classified as "restricted-use", can be used within food establishments when used in bait stations placed at least 1.2 m from the ground in areas where food processing or preparation does not occur.

An imidacloprid sprayable bait, Maxforce® Fly Spot, has recently become commercially available. It contains 10% imidacloprid, 0.1% Z-9-tricosene, Bitrex®, and inert ingredients. This formulation still maintains the advantages of traditional scatter baits, while eliminating some of the disadvantages of the currently available products. Once applied, Maxforce® Fly Spot bait dries clear and the label allows for application within food establishments when the facility is not in operation.

Our objectives were to compare the effectiveness of the new sprayable bait in relation to the two most commonly used dry scatter baits. In addition, we compared the performance of the imidacloprid sprayable and granular baits in a controlled field environment and tested the imidacloprid sprayable bait impregnated in cords.

Materials and Methods

Insects. The Horse Teaching Unit (HTU) strain of house flies, *M. domestica* L., reared at the University of Florida in Gainesville was used for all experiments. Larvae were reared on a diet medium, modified from Hogsette (1992), containing 3 liters wheat bran, 15 ml methyl paraben, 1.5 liters water, and approximately 200 g (250 ml) dairy calf feed (Calf Manna® pellets, Manna Pro Corp., St. Louis, MO). All developmental stages were held at 26 ± 1°C and 55% RH with a 12:12 (L:D) photoperiod. Adult flies emerged within in screened rearing cages and were provided granulated sugar, powdered milk, and water *ad libitum*.

For all assays, adult house flies (3-5 d old) were aspirated from the screened rearing cages using a handheld vacuum with a modified crevice tool attachment. Flies used for the laboratory assays were placed into a 5°C environment for 5 min to subdue activity. Flies were then placed on a chilled aluminum tray, sexed, and counted. House flies used for field cage assays were not subdued, but were aspirated from the screened rearing cages and released directly into field cages.

Laboratory Arena Design. Arenas (31 x 25 x 21 cm) were constructed using PVC pipe (1.27 cm [1/2 in] diam) (Figure 3-1A). Rubber bands were used to establish five uniformly distributed cord positions along the length of the arena. Each position held one cord which was vertically attached to the rubber bands using paper clips. Five cords were used with all laboratory experiments: nylon (Braided, Multi-Purpose Braid 75 lb. load limit, Wellington Cordage LLC, Madison, GA), polypropylene (Braided, Multi-Purpose Rope – 56 lb. load limit, Wellington Cordage LLC, Madison, GA), cotton (Braided, Multi-Purpose Sash Cord – 28 lb. load limit, Wellington Cordage LLC, Madison, GA), manila (Twisted, Natural Rope – 108 lb. load limit, Wellington Cordage LLC, Madison, GA), and wool (Twisted, Natural Cord, Wooded

Hamlet Designs, Greencastle, PA). Arenas were enclosed with a transparent plastic bag (3716 cm2 [24 x 24 in], 1 mil poly, Uline, Waukegan, IL).

Field Cage Design. Cages (1.8 x 3.7 x 1.8 m) were constructed from PVC pipe (2.54 cm [1 in] diam) and enclosed with mesh screening (Outdoor Cage, #1412A, 18 x 14 mesh, Bioquip, Rancho Dominguez, CA). Black plastic sheeting (6 mil) was used to line the floor. A sampling stage, constructed of two vertical cinder blocks and an inverted storage bin (Palletote #1721, 37 liter, Rubbermaid, Winchester, VA), was placed in the center of the cage (Figure 3-1B). On top of the sampling stage there were two 994-ml (1 qt) chick waterers, one filled with 10% sugar water and the other with tap water, and a 60-ml plastic cup filled with 8 g of previously used larval house fly medium. The chick waterers provided enough sustenance for the duration of the test and the plastic cup was used as an attractant. The plastic cup was covered with a paper towel and sealed with a rubber band to prevent flies from ovipositing on the medium.

Fly Bait Comparisons. Three fly baits, 2 dry scatter baits and 1 sprayable bait, were applied to polystyrene Petri dishes (100 by 15 mm; Fisher Scientific, Pittsburgh, PA). The methomyl granular bait (Golden Malrin®, Methomyl 1.1%, (Z)-9-Tricosene 0.049%, Wellmark International, Schaumburg, Illinois; dose: 0.23 g/0.9 m^2) and the imidacloprid granular bait (Maxforce® Granular fly bait, Bayer CropScience, Kansas City, MO; dose: 30.17 g/0.9 m^2) were sprinkled on the Petri dish. The Imidacloprid sprayable bait (Maxforce® Fly Spot bait, Imidacloprid WG 10, Lab Code: 342/207-7, Bayer CropScience, Monheim am Rhein, Germany; dose: 0.45 g/0.9 m^2; rate: 0.12 g Pr/ml/0.9 m^2) was suspended in tap water, sprayed on the Petri dish bottom using an airbrush (Paasche, Type H, Chicago, IL), and allowed to dry in a fume hood prior to being placed in the arena. Bait dishes were placed on the bottom rubber bands in the center of the arena. A separate arena with an untreated Petri dish was used as the control.

Cords in these experiments served only as resting positions for the flies, they were untreated and hung in the same configuration for all repetitions: (left to right: position 1 = cotton; 2 = wool; 3 = manila; 4 = polypropylene; 5 = nylon).

Groups of 50 female flies were placed within each arena and a 10% sugar water solution was provided *ad libitum*. Mortality was recorded at 1, 3, 5, and 24 h. Flies were considered dead if they were unable to stand or fly. Each experiment was run in the laboratory (30 ± 1ºC) under continuous light and replicated three times.

The two imidacloprid baits were evaluated in the field cages. Treatments consisted of two plastic lattice squares (0.19 m^2) treated with imidacloprid granular bait, imidacloprid sprayable bait, or tap water (control). Treatments were applied on only one side of the lattice at the same rates as the laboratory fly bait comparison assays. The imidacloprid granular bait was mixed with tap water (1.44 g: 1 ml) and painted on. The tap water (3.78 ml/0.9 m^2) and imidacloprid sprayable bait (0.12 g Pr/ml/0.9 m^2) were sprayed on using an airbrush. All treatments were allowed to thoroughly dry outdoors in the open air before being hung on the ceiling PVC pipes using cable ties. Each lattice square was placed medially along the length of the cage, approximately 0.5 m away from each side of the sampling stage and positioned so that the treated surfaces of the lattice squares faced opposite directions.

Depending on fly availability, 27.5 – 35 ml (9.8 ± 1.8 flies/ml) of flies were released into each cage. After a 1-h acclimation period, pre-treatment fly counts were taken. Before fly counts were taken, the operator walked three laps around the interior of the cage to disturb flies from their resting positions and to recover any dead flies from the cage floor. Four consecutive fly counts were then taken from the outside of the cage 1 min after exiting. All flies that landed on the sampling stage, chick waterers, and plastic cup attractant were counted. Treatments were

then hung within the cages and post-treatment fly counts were taken at 1 and 24 h using the same method described above. After the initial 24 h evaluation, treatments were aged in the elements for two weeks, at which point residual effectiveness was re-evaluated as described above. Three replicates were performed at each treatment age (0 and 2 wk).

Bait-Treated Cords. Five laboratory arenas were organized into a 5 x 5 Latin square design, blocking for treatment position, and a sixth arena was used as the control. Each treatment consisted of a cord (15.2 cm length, 0.6 cm diam) impregnated with a 2.5% solution of imidacloprid sprayable bait. The imidacloprid solution was prepared by combining 25 g of the formulated insecticide with 100 ml of tap water. Cords were impregnated by dipping for ~2 sec in the insecticide solution and were then allowed to dry on aluminum foil covered trays in a fume hood prior to being placed into the arenas. The control arena had no treated cords and had the same cord configuration as the fly bait comparison bioassay described above.

Laboratory tests were conducted with groups of 60 female flies/arena. Flies were provided a 10% sugar water solution *ad libitum*. Morbidity (knockdown) was recorded at 2-5 h post-treatment and mortality was recorded at 24, 48, and 72 h. Flies were considered knocked down if they did not move when touched at the 2-5 h recordings. Flies that were unresponsive to touch at the 24, 48, and 72 h recordings were considered dead. Each experiment was run under continuous light in the same laboratory conditions as described above and replicated twice.

In the field cages, treatments consisted of two long (0.9 m) and eight short (0.6 m) lengths of imidacloprid-impregnated wool cords. Each cord was treated in the same manner as the laboratory experiments, except the cords were dipped and soaked for 1 min prior to drying. Flies were released and fly counts were taken in the same manner as the imidacloprid bait field cage experiments. Cords were hung vertically from the mesh ceiling using paper clips in specific

locations, which remained constant throughout the experiment (Figure 3-1C). Post-treatment sampling counts were done at 24 and 48 hrs. After the initial 48 h evaluation, treatments were aged in the elements for four weeks, at which point residual effectiveness was re-evaluated as described above. Three replicates were performed for each treatment age (0 and 4 wk).

Data Analysis. All analyses were done using a one-way analysis of variance with JMP IN (SAS Institute 2005). For the fly bait comparison and the bait-treated cord experiments, percent morbidity (bait-treated cords) and mortality data were arcsine square root-transformed and analyzed for each time interval. For the field cage experiments, percent fly count reductions were calculated from the control fly counts. Fly count reductions and mortality data (number of dead flies recovered from cage floor) were then analyzed for each treatment age (0 and 2 wk for the imidacloprid comparisons; 0 and 4 wk for the bait-treated cord experiments) (Conover and Iman 1981). Means for all analyses were separated using the Student's T test or the Student Newman Kuels (SNK) method ($\alpha = 0.05$).

Results

In all laboratory experiments, flies did not fully recover from chilling until roughly 1 h after entry into the arenas. Flies first contacted the baited Petri dishes in the bait comparison experiments approximately 35 min post recovery in the following order: imidacloprid granular bait, imidacloprid sprayable bait, methomyl granular bait. Initial fly contact on the treated cords in the bait-treated cord experiments was not observed.

Fly Bait Comparisons. The imidacloprid granular and the imidacloprid sprayable baits had higher fly mortality than the methomyl granular fly bait at 3 h, but by 24 h the methomyl granular bait had the highest overall mortality (Figure 4-1). At 24 h, fly mortality with the imidacloprid sprayable bait was not significantly different from mortality with either the imidacloprid granular or the methomyl granular fly baits, but significant difference in fly

mortality did exist between the methomyl and the imidacloprid granular fly baits. All treatments were significantly different than the control fly mortality at all observations ≥ 3 h.

In the imidacloprid field cage experiments, no differences were seen between either treatments with fresh or aged cords (Table 4-1). Both treatments had >35% fly count reductions at 24 h and >70% fly count reductions by 48 h with fresh cords, but fly count reductions did not exceed 8% with aged cords for either treatment. A fly count increase was observed with the imidacloprid granular treatment at 48 h with aged cords. The number of dead flies collected in the treatment cages was significantly different from the control with fresh cords, but no differences were observed between the aged treatments and controls 2 wk post-treatment.

Bait-Treated Cords. Morbidity increased on a time-dependent basis until approximately 3-4 hours post-treatment, at which time flies recovered from being knocked down by all of the imidacloprid bait-treated cords except for the cotton cord (Figure 4-2). Flies exposed to all imidacloprid bait-treated cords had knockdown recovery by 24 h. All cords caused significantly more mortality than the control cords at every 24 h recording (Figure 4-3). Beyond 24 h, mortality with the imidacloprid bait-treated nylon, cotton, and wool cords increased more sharply than the mortality caused by the bait-treated manila and polypropylene cords. The imidacloprid bait-treated wool cord caused the highest overall fly mortality (74%). All other cords resulted in house fly mortalities <60% with the imidacloprid bait-treated polypropylene cord showing the lowest overall fly mortality (25%).

In the field cages, the imidacloprid bait-treated cords caused >87% fly count reductions by 24 h with fresh and aged cords (Table 4-2). The aged bait-treated cords fly count reductions decreased by ~6% by 48 h, whereas fly count reductions increased by ~6% with the fresh cords.

The number of dead flies collected was significantly different than the control at 24 and 48 h for both fresh and aged cords, except for the 48 h recording with the aged cords.

Discussion

When insecticides are used for house fly control, most users expect to see satisfactory results (i.e. dead flies) within hours and markedly reduced populations within 1-2 days. Thus, an effective fly bait will attract flies quickly and cause high mortality within a relatively short period of time. In our bait comparison experiment, flies contacted the imidacloprid baits sooner than they contacted the methomyl bait, which may have been a contributing factor to the higher fly mortality at 3 h with the imidacloprid baits than with the methomyl bait. However, the higher fly mortality with the methomyl bait after 24 h suggests that methomyl may be a more potent, although slower acting, active ingredient.

Other studies comparing imidacloprid and methomyl baits have also shown the same mortality trends we observed in flies exposed to technical and bait formulations of imidacloprid and methomyl (White et al. 2007). In those experiments, White et al. observed up to 50% of the flies that were knocked down by imidacloprid formulations recovered. They hypothesized innate characteristics, independent of resistance mechanisms, may make some flies tolerant to neonicotinoids. We observed knockdown recovery in the bait-treated cord experiments with all cords, but no recovery was seen in the house flies exposed to any of the baits we tested in the bait comparison experiments. Recovery may have occurred in these experiments, but was not observed because recordings were not taken between the 5 h and 24 h recordings. Flies that were knocked down were not isolated from the arena in our experiments and could have received a second dosing before having the opportunity to fully recover.

Differences in cord material or treatment application technique may have also attributed to fly recovery in the bait-treated cord experiments. Cord saturation is dependent on the cord

composition and may have lead to sublethal dosing. We observed that cord composition varied between the types of cords we used, and even among individual cords. Distribution of oils and other materials on the surface of each cord make it difficult to have the bait uniformly distributed over the surface of the cord. When bait is sprayed onto a solid surface, such as the Petri dish, a precise amount of bait remains on the surface after the water evaporates. However, when a cord is dipped into a bait solution, the bait may be absorbed deep into the fibers, disperse throughout, or pool in areas on the cord. Thus, some bait may not be available for flies to contact. This is evident when hand-dipping cords in dyed insecticides materials, such as in an indoxacarb wettable granule (WG) solution, which is grayish-brown in color. Despite being fully submerged in the insecticide solutions, some of the cord often remains its natural color, apparently void of any bait. Once the same cords are allowed to completely dry and are removed from the drying trays, brown staining surrounds were they once lay indicating that some of the insecticide may be lost during the drying process as well.

It is undetermined if knockdown recovery occurred in our field cage experiments. If flies are knocked down in the field, natural enemies may prey upon them before they are able to fully recover. We observed knocked-down flies being preyed upon by ants, spiders, and lizards. Others, undoubtedly, became victim to desiccation after being knocked down. Barson (1987) found that flies knocked down by methomyl in the field often lost their ability to fly, but still had the ability to reproduce. White et al. (2007) commented that 10% of the flies knocked-down by imidacloprid in their laboratory studies fully recovered and resumed normal behavior when protected from a second exposure to imidacloprid. The inability to fly would make flies more vulnerable to predation by natural enemies. However, if reproduction is still occurring, it will be detrimental to any fly control program because of a fly's prolific reproductive capabilities. Field

studies to determine the effect of knock down and recovery on a fly management program would be beneficial.

Insecticide-impregnated cords have been used extensively in the past to control flies and have recently been examined using new insecticides not yet registered for use against house flies (*Unpublished,* Chapter 3). In those experiments, indoxacarb and fipronil were more effective on wool cords than any other natural and synthetic cords tested. Wool cords also showed higher efficacy than the other natural and synthetic cords tested when treated with the imidacloprid sprayable bait. Exact LT_{50}'s were not determined in these experiments because of the knockdown and subsequent recovery observed, but based on Figure 4-3, we estimate that 50% of the flies died after approximately 60 hours (2.5 d) with the wool cord. With such a long period to reach 50% fly mortality, imidacloprid-treated cords were slower acting than any of the fipronil- or indoxacarb-impregnated cords previously tested. However, in the field cages, the imidacloprid bait-treated cords reduced fly counts by 80% in 24 h. The high lipid content of wool cord may facilitate the transfer of insecticide through the cuticle of the house fly, but this does not explain the differences in results between the laboratory and field assessments. Differences in the rate of cords per cage area may explain the differences in the laboratory and field results. The cords in the field cages were hung at a rate of 9.1 m of cord/9.3 m^2 area based on the recommended rate of the insecticide cords used in the 1950's (Fehn 1958, Smith 1958, Weinburgh et al. 1961). The rate in the laboratory arena was comparatively much lower, 0.02 m of cord/m^2 vs. 1.0 m of cord/m^2. This rate appears to be quite high and may vary between different cord/insecticide combinations. Additionally, the aforementioned predation and desiccation of the knocked down flies probably was significant factor contributing to the rapid fly count reductions in the field cages.

When comparing the imidacloprid bait-treated cords and the imidacloprid bait-treated lattice squares, the bait-treated cords were more effective. The lattice squares did not reduce the fly counts to any significant degree after being aged 2 weeks, but the imidacloprid bait-treated wool cords had good fly count reductions even after being aged 4 weeks. The plastic lattice squares were selected as a treatment surface to represent the material found on many portable toilets, latrines, or dumpsters. Bait treatments on this type of surface are very vulnerable to environmental conditions because the material does not allow the bait to penetrate as in the cord treatments. Damp conditions in the mornings and unexpected precipitation (6.5 cm) that occurred between evaluation intervals washed away most of the bait product from the lattice squares. With the imidacloprid granular bait application, the lattice squares were almost completely void of the red dye following these moisture events and red staining was seen on the cage floor. We assume that the imidacloprid sprayable bait was also washed off the lattice squares given the results of the fly count reductions and the dead flies recovered, but was not observed because the bait has no color. The bait-treated cords were exposed to 3.5 cm less precipitation than the bait-treated lattice squares, which may have also affected the bait available on the cord. When dipping cords in a bait solution, the bait is absorbed in between individual cord fibers and even deeper into the core of the cord, making the bait more protected from environmental conditions. When the cords are then subjected to these moisture events, the bait may concentrate in specific areas of the cord (such as the cord end) instead of completely leaving the cord as seen with the lattice. Additionally, flies prefer to rest on cords and probably receive a larger dose of insecticide in this manner as compared to when they land on the flat surfaces of the lattice. When a fly lands on a flat surface they are exposed only to the precise amount of

toxicant that absorbs through their tarsi or is imbibed; however, when resting on cords, their thorax and abdomen are also brushed by the treated cord fibers.

In conclusion, the imidacloprid sprayable bait was found to be as effective as the traditional commercial scatterbaits compared in this study. Its unique formulation and less restrictive product label allow this bait to be used in areas where other fly baits are prohibited. Unless a more rain-fast formulation becomes available, the imidacloprid sprayable bait will need to be reapplied frequently in areas with high moisture or precipitation especially when applied to non-absorbent surfaces such as portable latrines or dumpster lids. The bait's potential effectiveness in insecticide-impregnated cords needs further investigation due to differing laboratory and field results. Regardless, this new imidacloprid sprayable bait should prove to be a very useful tool in any fly management program.

Table 4-1. Number of dead and percent fly count reduction in relation to control fly counts of house flies exposed to imidacloprid bait-treated lattice squares in field cages.

		% Fly Count Reduction ± SEM‡		# of Dead Flies†‡	
Treatment Age	Treatment	1 h	24 h	1 h	24 h
0 Weeks	Imidacloprid granular bait	47.1 ± 6.3a	70.9 ± 4.4a	36.0 ± 10.0a	117.0 ± 9.5a
	Imidacloprid sprayable bait	36.6 ± 20.5a	80.2 ± 4.7a	36.3 ± 2.0a	113.0 ± 10.1a
	Control			0.3 ± 0.3b	1.7 ± 0.7b
2 Weeks	Imidacloprid granular bait	0.8 ± 8.2a	-3.8 ± 20.1a	1.0 ± 0.6a	19.7 ± 11.2a
	Imidacloprid sprayable bait	7.6 ± 10.8a	6.3 ± 19.5a	0.7 ± 0.7a	14.0 ± 11.6a

† Mean number of individuals recovered from cage floor.

‡ Means in a column, within the same treatment age, followed by the same letter are not significantly different ($P > 0.05$; Student's T test or Student-Newman Kuels Method)

Table 4-2. Number of dead and percent fly count reduction in relation to control fly counts of house flies exposed to imidacloprid bait-treated cords in field cages.

		% Fly Count Reduction ± SEM		# of Dead Flies†	
Treatment Age	Treatment	24 h	48 h	24 h	48 h
0 Weeks	Bait-Treated Cords	90.4 ± 4.1	96.8 ± 3.2	97.7 ± 17.2a	114.3 ± 19.8a
	Control			12.7 ± 4.6b	19.7 ± 7.7b
4 Weeks	Bait-Treated Cords	87.9 ± 0.9	82.4 ± 14.7	50.7 ± 14.0a	69.3 ± 23.7a
	Control			8.7 ± 3.8b	13.0 ± 4.7a

† Mean number of individuals recovered from cage floor. Means in a column, within the same treatment age, followed by the same letter are not significantly different (P > 0.05; Student's T test)

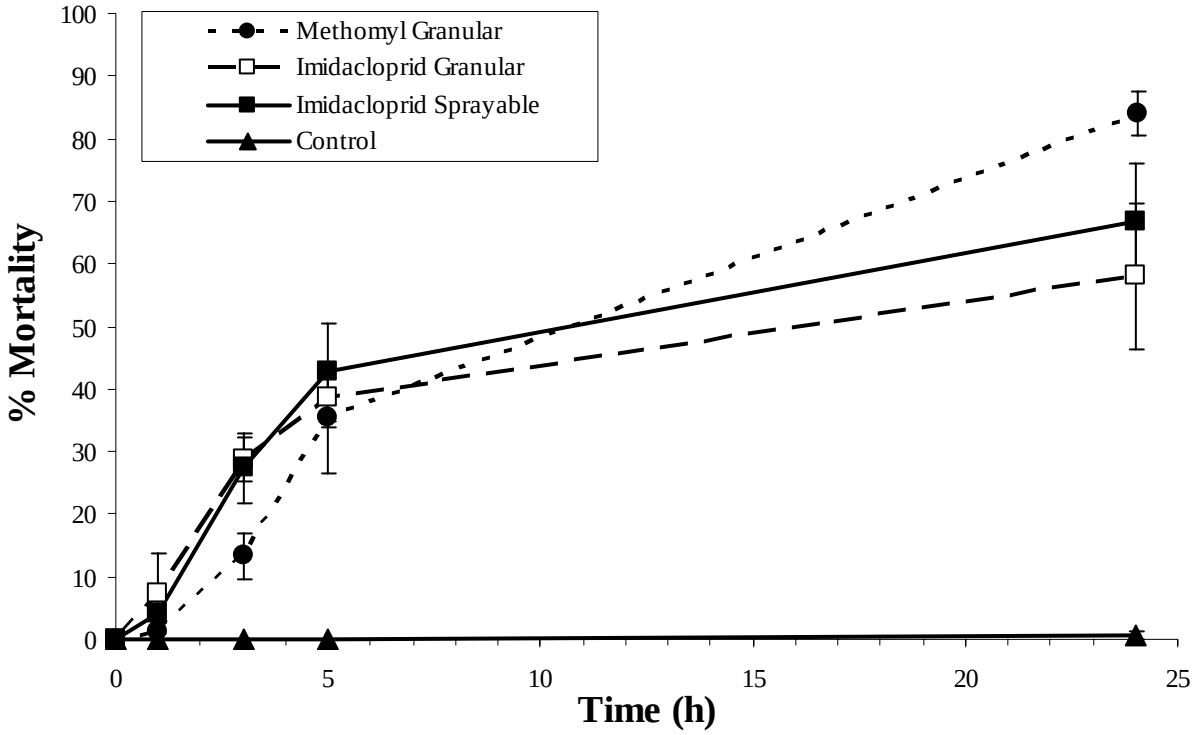

Figure 4-1. Mortality of female house flies exposed to imidacloprid and methomyl granular scatter baits and a sprayable imidacloprid bait.

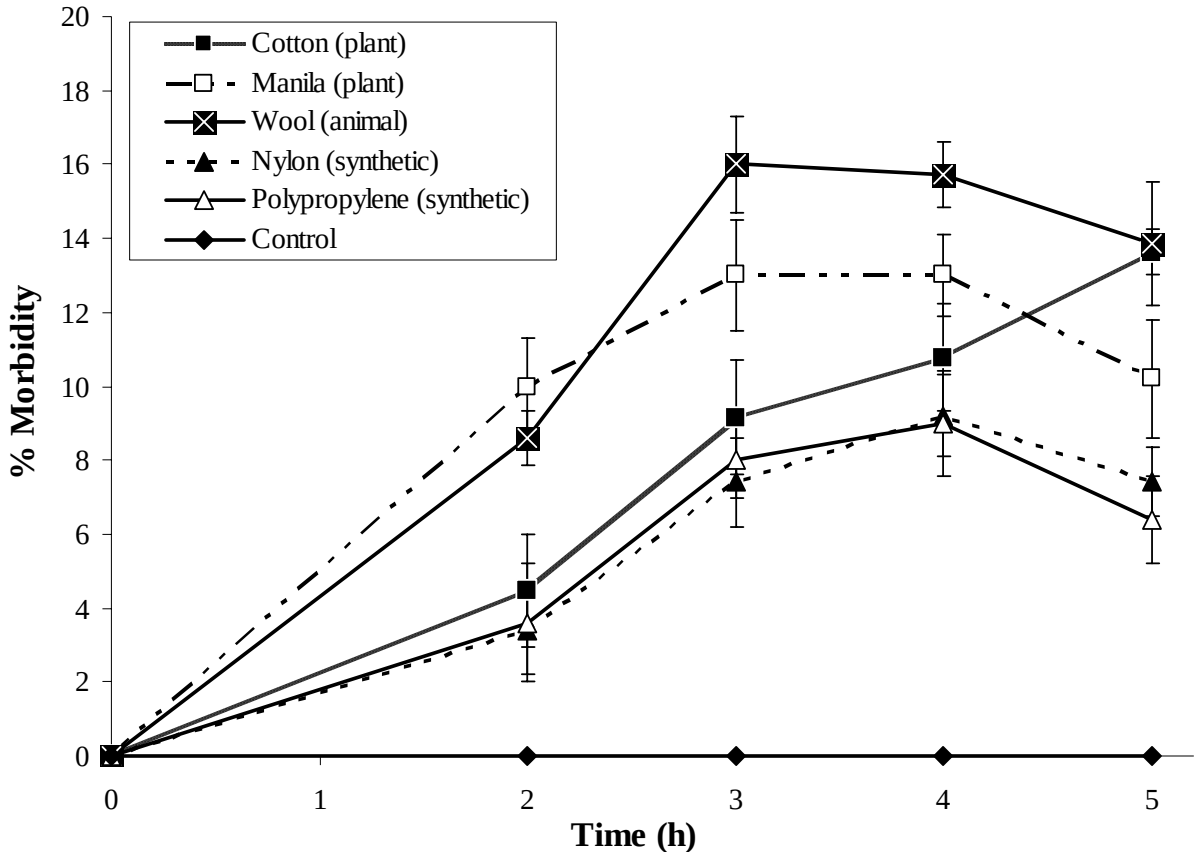

Figure 4-2. Morbidity (knockdown) of female house flies exposed to natural and synthetic cords dipped in a 2.5% solution of imidacloprid sprayable bait.

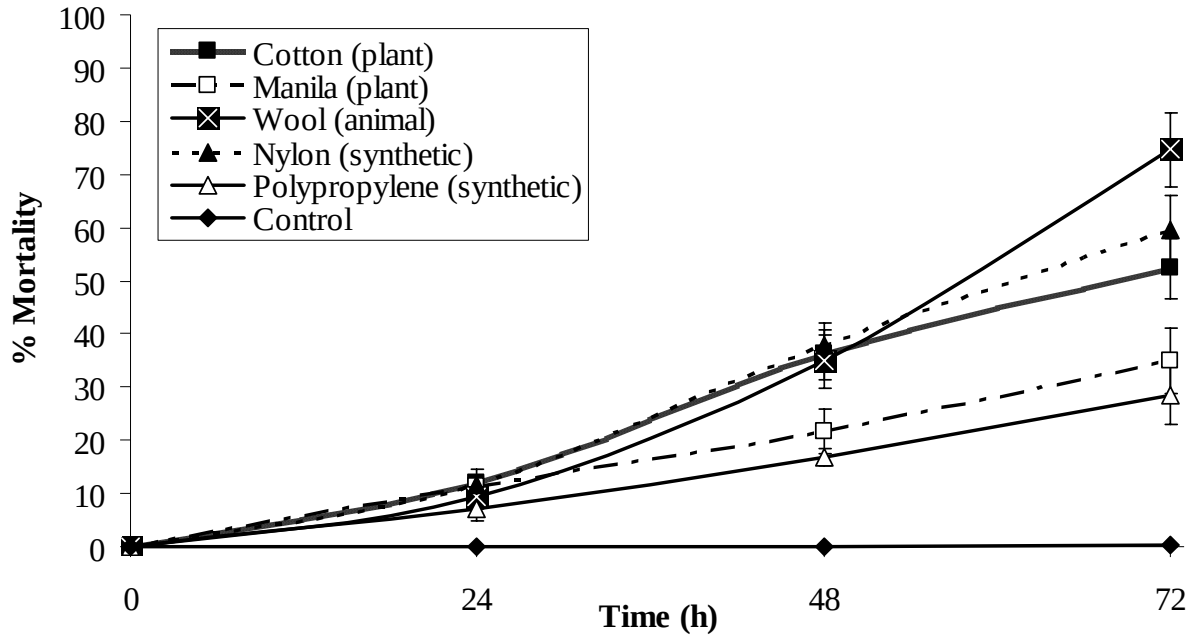

Figure 4-3. Mortality of female house flies exposed to natural and synthetic cords dipped in a 2.5% solution of imidacloprid sprayable bait.

CHAPTER 5
SUMMARY AND CONCLUSIONS

House flies are often found in tremendous numbers in situations where U.S. troops are most frequently deployed, such as areas stressed by conflict or natural disasters. House flies are nuisance pests that decreases troop morale and they pose a health risk to deployed troops that may disrupt mission objectives if diseases that are associated with their presence stress the health care systems. The main objective for this research was to evaluate insecticide-impregnated cords and sprayable fly bait as new methods to control the house fly and provide usable information to the DOD that will help protect the deployed war fighter.

First and foremost, house flies can be controlled using insecticide-impregnated cords and sprayable fly bait and their use would benefit agricultural, urban, and military fly management programs. Insecticide-impregnated cords and sprayable fly baits are both very easy to use products. Impregnated cords can be hung using a staple gun or other similar method and sprayable bait is mixed with water and sprayed onto any surface. Impregnated cords can be removed and relocated quickly, which may be beneficial in relatively mobile troop deployments or in situations were resistance is suspected. Sprayable baits can be removed with simple water wash down and easily reapplied when needed.

One of the most interesting findings in this research is the further understanding in insecticide-impregnated cord toxicity. Previous insecticide-impregnated cords were made exclusively of cotton because it was cheap, durable, and relatively attractive to house flies, however, the cotton cords in our experiments were the least effective cord. Wool cords consistently showed they were more effective than any of the other cords we evaluated despite the fact that they were not the most attractive cord to the house flies. House flies were most attracted to the highly organic manila cord and preferred to rest on it more than any other natural

or synthetic cord evaluated. The wool cord was the only animal-based cord impregnated with insecticides and possibly gave it a distinct advantage over the other cords because it is naturally coated with oil, which probably helped facilitate insecticide transfer through the fly cuticle.

Two fly scatter baits, Maxforce® Granular and Golden Malrin® fly bait, are both listed on the DOD pesticide contingency list for house fly control but both have limitations. Only one can be applied in food service areas and both can stain materials and equipment which can compromise camouflage and substrate appearance. Eliminating these disadvantages, while maintaining bait efficacy, would appear to provide advantages to a fly management program and benefit the deployed war fighter. The new sprayable imidacloprid bait, Maxforce® Fly Spot, is as effective as the two previously mentioned scatter baits in the laboratory and as effective as its counterpart, Maxforce® Granular, in the field. Its unique formulation allows it to be used within food serving areas and it will go unnoticed because it dries clear. Unfortunately, like the other bait products, environmental factors, such as rain, decrease the efficacy over time. Residual efficacy did improve when the bait was applied to the cords rather than the non-absorbent plastic surfaces often found on latrines and dumpsters.

We anticipated that the research completed here would provide some information that could be further used to develop future products that could be benefit the DOD. The insecticides evaluated in the impregnated cord studies are both non-registered for house fly control. House flies have shown little to no resistance towards fipronil and indoxacarb and both insecticides appear to be very effective against this pest. At this time, no information has been obtained on whether or not the manufacturers of fipronil or indoxacarb are seeking registrations for these products to control flies. However, a Colorado company has informed me of their interest in indoxacarb-impregnated cords and has begun conversations with DuPont® regarding further

research into this type of product. The sprayable imidacloprid fly bait is not currently listed on the DOD pesticide contingency list but it received its EPA registration in 2006 and became commercially available in mid 2007. A formal request will be submitted to the Armed Forces Pest Management Board this summer to request that it be assigned a National Stock Number (NSN) and be placed on the DOD pesticide contingency list. If successful, Maxforce® Fly Spot will be readily available to the deployed war fighter for use in their fly management programs.

APPENDIX A
REVIEW OF INSECTICIDE-IMPREGNATED CORDS

The use of insecticide-impregnated cords to control house flies was first tried with DDT in 1947 (Baker et al.). By the early 1950's, impregnated materials for fly control became increasingly common (Pimentel et al. 1951). Insecticide-impregnated cords were being used on dairies, at rural residences, military mess halls, state fairs, and state prisons with great success (Kilpatrick 1955, Maier and Mathis 1955, Soroker 1955, Kilpatrick and Schoof 1956). Commercial Fly-Cords distributed by Fly-Cord Inc. (Savannah, Georgia) were widely available and used by 1957 (Fehn 1958, Smith 1958). These cords were considered the treatment of choice for use in buildings housing animals because of the economy and efficiency (Fay and Kilpatrick 1958).

Fay and Lindquist (1954) recognized that impregnated cords offer only a small percentage of the surfaces available for flies to rest. Exploiting the factors which enhance a fly's attraction to a particular cord would subsequently lead to higher mortality on impregnated cords. They found that cord type, thickness, and color significantly influenced a fly's attraction to a particular cord. Sisal and cotton cords were more attractive than jute or wool cords. Cord attractiveness increased with cord diameters between 3/64" and 7/16". Flies preferred red and black cords over blue, yellow, green, or white cords. No preference was evident between vertically or horizontally hung cords.

Although several other organophosphate insecticides have been evaluated for their effectiveness, all provided satisfactory results. However, only one cord was available commercially (Kilpatrick and Schoof 1959, Gratz et al. 1964, Rabari and Patel 1976). The commercial Fly-Cord was a 3/32" diameter, red cotton cord impregnated with 13.79% parathion and 3.54% diazinon (Fehn 1958, Smith 1958). The cord was supplied on a reel containing 300

feet; each linear foot of cord contained 75-100 mg of parathion (Youngblood 1960). The manufacturer recommended a rate of 30 linear feet of Fly-Cord per 100 square feet of floor area in locations were adult flies congregate (Fehn 1958, Smith 1958). Cords were normally hung about three feet apart using staple guns or simply tied on to the structure (Fehn 1958, Youngblood 1960). If multiple competing resting surfaces were available to the flies, most applicators increased the amount of impregnated cords in the area. In places where flies were not seen resting or where conditions were unsuitable (i.e. areas with drafts), no cords were placed.

The use of impregnated-cords was an attempt to find new methods to reverse the resistance associated with residual spraying of chlorinated hyrdrocarbons such as DDT (Keiding and Jespersen 1986). Fly-Cords offered an easy-to-use control method that restricted and concentrated a residual insecticide. This method lowered the selection pressure for resistance because flies which avoided contact with the cords diluted the remaining population of resistant individuals (Keiding and Jespersen 1986). Other methods, such as paint-on baits, non-residual space sprays, and combining baits and larvicides, were evaluated and determined effective (Keiding and Jespersen 1986). Paint-on baits and non-residual space sprays are widely available and used today in the United States.

No commercially available insecticide-impregnated cord products are currently available in the United States. This is partially because the main active ingredients, parathion and diazinon, are not registered for filth fly control. In addition, the use of selective insecticidal baits has become increasingly popular. In Denmark, the use of impregnated cords was abandoned because of newer construction techniques that allowed for more ventilation in the animal shelters and more effective residual sprays became available (Keiding and Jespersen 1986). The World

Health Organization and the United States Military continue to recommend the use of insecticide treated or impregnated cords (Rozendaal 1997, AFPMB 2006)

APPENDIX B
REVIEW OF FLY BAITS

Insecticidal baits have long been used for fly control. One of the original bait formulations contained either 1-2% formaldehyde or sodium arsenite mixed with milk or sugar water (Keiding 1976). Residual insecticides have been mixed with sugar to make them more attractive to flies and serve as a type of bait, but this type of mixture is often not as effective as baits specifically formulated to attract flies. Today's fly baits are loaded with many different attractants including pheromones, sugars, and other substances that specifically attract house flies. Most of these fly baits are formulated as either dry scatter baits, but many also come in easy-to-use bait station devices. Some of the dry scatter baits can be mixed with water and painted on a surface.

Insecticidal baits have many advantages over other chemical control methods. Baits are relatively inexpensive, usually have a longer storage shelf life, are more attractive to flies than other chemical control methods, and are easier to work with in field environments (Gahan et al. 1954, Darbro and Mullens 2004). The dry scatter baits simply get scattered on the ground in the infested areas or placed within a bait station while bait station devices normally only need to be opened and hung in infested locations. Keiding (1976) considered baits less likely to select for resistance than residual sprays. He was most likely referring to the physiological resistance seen in many of the organophosphate and carbamate insecticides at that time. Today, many insects, including flies, have been shown to develop behavioral resistance to baits (Darbro and Mullens 2004).

Fly baits do have disadvantages. Dry scatter baits do not target fly resting areas unless they can be painted on and this type of application often leads to stained surfaces because the U.S. Environmental Protection Agency (EPA) requires the scatter bait granules to be dyed to distinguish them from other non-toxic materials. Staining can also occur when these granules

become wet by rain and bleed onto the surrounding surface, which may be considered unsightly for the user. Bait station devices are also degrade rapidly in environments with intense sun or precipitation. Dry scatter baits also need to be replaced frequently in some areas when granules become covered by manure or other debris such as garbage (Barson 1987).

As with any chemical insecticide, label restrictions can be disadvantageous (although necessary in most cases) and limit the needs of the applicator. For example, the two most widely used dry scatter baits in the United States are Maxforce® Granular fly bait and Golden Malrin® fly bait. Maxforce® Granular is an imidacloprid-based bait (neonicotinoid class) containing the fly attractant (Z)-9-tricozene, the bittering agent Bitrex®, and other attractants and inert ingredients. Golden Malrin® contains 1.1 % methomyl (carbamate class), 0.049 % (Z)-9-tricosene, as well as other attractants and inert ingredients, and is one of several methomyl-based scatter baits available. Maxforce® Granular has a more restricted label than that of Golden Malrin® because its label restricts its use in food establishments. Golden Malrin®, despite being the only carbamate-based insecticide not classified as a "restricted-use" insecticide, can be used within food establishments when used in bait stations placed at least 1.2 m from the ground in areas where food processing or preparation does not occur.

Recently, a new fly bait has become commercially available that may offer advantages over some of the other current fly baits available. Maxforce® Fly Spot bait contains 10% imidacloprid, 0.1% Z-9-tricosene, Bitrex®, and inert ingredients. Once applied, Maxforce® Fly Spot bait dries clear and the label allows for application within agricultural livestock production facilities and serving areas of food establishments when the facility is not in operation.

APPENDIX C
REVIEW OF INSECTICIDES EVALUATED

Fipronil

Fipronil is a phenylpyrazole insecticide that was first registered in the United States in 1996 (Connelly 2001). It is used to control termites, ants, roaches, fleas, ticks, and various other agriculture and turf pests. No fipronil products are currently registered for house fly control.

Fipronil causes mortality by contact and ingestion (Vargas et al. 2005). Insects exposed to fipronil show extreme neural excitation that eventually leads to insect paralysis and death. Death is caused by the disruption of the normal passage of chloride ions through the γ-aminobutyric acid type A (GABA) receptor system of insects (Scharf et al. 2000). Hainzl et al. showed fipronil to have a tighter binding affinity toward insect GABA-regulated chloride channels over mammalian receptors (1998). Fipronil-sulfone, an important active metabolite of fipronil, was also found to block the glutamate receptors in cockroaches (Zhao et al. 2004). Glutamate-gated chloride channels are only found in invertebrate systems at skeletal neuromuscular junctions of both the peripheral and central nervous system (Raymond and Sattelle 2002, Scharf 2003). The unique quality of fipronil to affect two target sites makes it a highly selective insecticide and potentially important factor limiting the development of detectable resistance (Zhao et al. 2004).

To date, resistance to fipronil appears to remain at low levels or even be non-existent in house flies (Scott and Wen 1997, Scott et al. 2000, Kristensen et al. 2004). Low levels of cross-resistance have been reported in multi-resistant house flies and attributed to monoxygenase-mediated detoxification, decreased insecticide penetration, and target site mutations (Wen and Scott 1999, Liu and Yue 2000, Kristensen et al. 2004). Resistance surveys in New York found house flies susceptible to fipronil even at LC_{99} levels (Scott et al. 2000).

Indoxacarb

Indoxacarb (DPX-MP062) is an oxdiazine insecticide that was first registered in the United States in 2000 (EPA 2000). It is a 75:25 mixture of the active S-isomer (DPX-KN128) and the inactive R-isomer (DPX-KN127). A less effective formulation (DPX-JW062) contains a 50:50 mixture of the two stereoisomers. Indoxacarb was originally formulated to control lepidopteran pests of fruits and vegetables, but newer registrations include cockroach, mole cricket, and fire ant baits. It is not currently registered for house flies.

Indoxacarb is considered an organophosphate replacement and designated as a "reduced-risk" insecticide by the EPA. It is a pro-insecticide that must be biochemically converted to a toxic decarbo-methoxyllated metabolite (Dias 2006). Toxicological effects are dependent on the conversion of the inactive metabolite to its toxic form within the insect body. In mammals, indoxacarb metabolites are rapidly excreted; whereas in insects, indoxacarb is rapidly converted by an esterase and amidase into DCJW, which is the more insecticidally active metabolite. Insects exposed to lethal doses of indoxacarb experience impaired nerve function, feeding cessation, paralysis, and eventually death. Indoxacarb poisoning occurs through contact or ingestion and it works by blocking the sodium channel of the insect nervous system. This mode of action is distinct from other insecticides that target the sodium channels of insects (DDT, pyrethroids) because DCJW disrupts the sodium channels without modifying the activation or deactivation kinetics (Lapied et al. 2001). It works by blocking the channel pore, and prevents normal sodium ion flow.

Because indoxacarb is a new chemistry, not much work has been done on insecticide resistance. Shono et al. (2004) selected house flies that had >118-fold resistance in as little as three generations and concluded that the resistance mechanism was associated with a major

factor on autosome 4 and a minor factor located on autosome 3, both of which are not linked to any resistance mechanisms previously described.

Imidacloprid

Imidacloprid is a chloronicotinyl nitroguanidine (neonicotinoid) that was first registered in the United States in 1994 (NPTN 1998). It is used to control a wide variety of agricultural, urban, public health, and veterinary pests and is estimated to account for 11-15% of the total global insecticide market (Tomizawa and Casida 2005). Several formulations are available for different treatment applications, only two are available for house fly control: Maxforce® Granular fly bait and Maxforce® Fly Spot bait. Both products are baits, but differ from one another by their formulation. Maxforce® Granular fly bait is a red granule that can be applied as a traditional scatter bait, within a bait station, or mixed with water and painted onto a surface. The Maxforce® Fly Spot bait, alternatively, is white wettable powder that is mixed with water and sprayed onto a surface. When the Maxforce® Granular fly bait is painted on a surface, or if the granules become wet, it stains the surface red, whereas the Maxforce® Fly Spot bait is clear and does not stain.

Imidacloprid kills insects through contact and ingestion by agonizing the nicotinic acetylcholine receptor (nAChR) (Fossen 2006). In house flies, imidacloprid is metabolized by oxidation to the "olefin" metabolite, which has the same toxicological activity as imidacloprid (Nishiwaki et al. 2004). Insects exposed to lethal doses of imidacloprid experience nervous system excitability, modified feeding behavior, and death. Imidacloprid is considered a selective insecticide because: (1) imidacloprid has a higher affinity for the insect nAChR's than mammalian nAChR's, and (2) there are more nAChR's located in the insect nervous system than what are found in mammalian systems (Yamamoto et al. 1995).

Resistance has yet to be reported in house flies (Gao et al. 2007), but it is well-documented in Drosophila (Daborn et al. 2001). Cross resistance has been observed in multi-resistant house flies (Wen and Scott 1997). Multiple resistance mechanisms are suspected in house flies. Monoxygenase-mediated detoxication seems to be a primary mechanism in some strains of house flies, but not in others (Wen and Scott 1997, Liu and Yue 2000).

Methomyl

Methomyl is a carbamate insecticide that was first registered in the United States in 1968 (EPA 1998). It is used to control a wide variety of agricultural, urban, public health, and veterinary pests. Several methomyl formulations are available, but the 1% fly bait formulation is the only one which is not classified as a restricted-use pesticide.

Methomyl causes mortality by contact and ingestion by inhibiting the acetylcholinesterase (AChE) enzyme, which occurs in the central nervous system. Methomyl binds to AChE and prevents it from binding to acetylcholine. This results in acetylcholine saturation at its neural receptor, which results in a dramatic increase in generation of nerve impulses. Insects exposed to methomyl show signs of hyperexcitability, convulsions, paralysis, and death. Decarbamoylation of AChE is rapid and, therefore, carbamates are considered reversible AChE inhibitors and recovery from sub-lethal poisonings can occur quickly (Yu 2007).

Little resistance to methomyl has been seen in house flies despite its frequent use and the high levels of resistance seen in house flies to other carbamates (Barson 1989, Webb et al. 1989, Scott et al. 2000, Darbro and Mullens 2004). Flies feeding on methomyl granules have been found to receive a super-lethal dose that may play a large roll in why resistance has not been as widespread (Price and Chapman 1987). Behavioral resistance, or bait aversion, has started to become more apparent. In 1989, Barson (1989) reported that 8% of the resistant flies were repelled by methomyl. In a study comparing the mortality of 35 field strains of house flies fed

methomyl in choice and no-choice tests, mortality decreased by nearly 30% when the flies were given the choice test (Darbro and Mullens 2004).

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BIOGRAPHICAL SKETCH

Jeffrey Conrad Hertz was born in 1976 in Peoria, Illinois. His parents, Marion Conrad "Butch" Hertz and Margaret Eloise "Weezie" Hertz (Hilton), raised him in Lewistown, Illinois. They moved to Bernadotte, Illinois where he continued to attend school in neighboring Lewistown until he graduated from Lewistown Community High School in 1994. He entered the United States Navy and reported to basic training at Recruit Training Command, Great Lakes, Illinois in November later that same year. Over the last 12 years, he served with the United States Marine Corps, at Naval hospitals, and most recently, he was assigned to the medical staff at the United States Capitol. He received his Associate of Science degree in medical laboratory technology from George Washington University, Washington D.C. in 2002 and his Bachelor of Science in interdisciplinary studies, majoring in biology from Mountain State University, Beckley, West Virginia in 2003. In 2004, he was selected as the very first enlisted Sailor selected to study entomology under the Medical Service Corps In-service Procurement Program (MSC-IPP). Upon graduation HM1 (FMF) Jeffrey Hertz will be commissioned to the rank of Lieutenant Junior Grade as a medical entomologist in the Medical Service Corps. He enjoys running and is an active member of Centennial Lodge #174 of Ancient Free and Accepted Masons located in Upper Marlboro, Maryland. He, his wife, Karina, and two children, Conrad and Kyra, are excited about their upcoming move to Jacksonville, Florida where he will be working at the Navy Entomological Center of Excellence.